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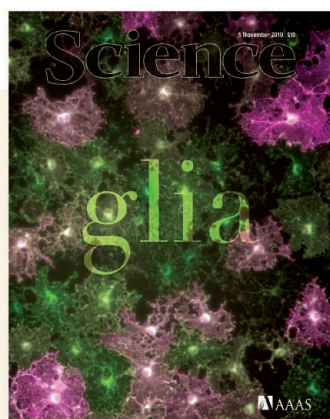
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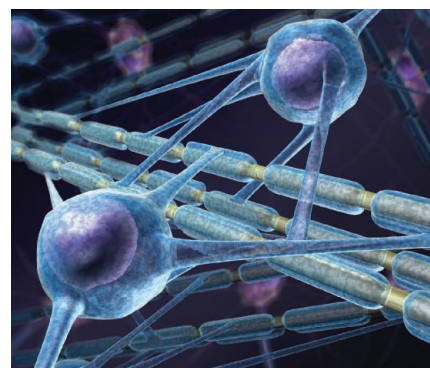
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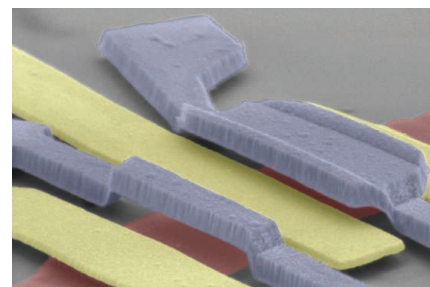
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10.1126/science.1191776

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Universality in the Evolution of Orientation Columns in the Visual Cortex

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10.1126/science.1197172

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Highlights From Our Daily News Coverage

Fish Oil No Foil for Alzheimer's

Study finds omega-3s do not stave off cognitive decline.

Bacteria Help Flies Select Mates

Microbes that live in and on the insects may foster the creation of new species.

Hellish 'Super-Earths' Likely Prevalent Throughout Our Galaxy

Study suggests that many extrasolar planets will be too hot for life.

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The Signal Transduction Knowledge Environment

EDITORIAL GUIDE: Focus Issue—Signals for Gene Expression

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RESEARCH ARTICLE: Rewiring of Transcriptional Regulatory Networks—Hierarchy, Rather Than Connectivity, Better Reflects the Importance of Regulators

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RESEARCH ARTICLE: DNMT1 Stability Is Regulated by Proteins Coordinating Deubiquitination and Acetylation-Driven Ubiquitination

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PERSPECTIVE: Posttranscriptional Regulation of PTEN Dosage by Noncoding RNAs

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PERSPECTIVE: Intracellular Delivery Strategies for MicroRNAs and Potential Therapies for Human Cardiovascular Diseases

M. A. Shi and G.-P. Shi

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REVIEW: Fibroblast Growth Factor Receptor Signaling Crosstalk in Skeletogenesis

H. Miraoui and P. J. Marie

Crosstalk of fibroblast growth factor signaling with other pathways may offer therapeutic strategies for skeletal dysplasias.

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N. H. Steneck

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Taken for Granted: Ain't Misbehavin'

B. L. Benderly

Research suggests that social structure, not personal ethics, determines the frequency of scientific misconduct.

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REVIEW: Mathematical Modeling of Molecular Data in Translational Medicine—Theoretical Considerations

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RESEARCH ARTICLE: Altered Functional Connectivity Within the Frontal Lobe of the Brain Is Associated with Variation in *CNTNAP2*

A. A. Scott-Van Zeeland et al.

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RESEARCH ARTICLE: Cholesterol Oxidation Products Are Sensitive and Specific Blood-Based Biomarkers for Niemann-Pick C1 Disease

F. D. Porter et al.

Oxysterols are biomarkers for diagnosis and drug treatment of a childhood neurological disease.

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THE GONZO SCIENTIST: Why Do Scientists Dance?

To cap off the 2010 "Dance Your Ph.D." contest, our reporter explores the history of science dances and asks the contestants how online geek fame has affected their lives.

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ADVANCING SCIENCE, SERVING SOCIETY



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Lifebelt for UK Science

WHEN THE UNITED KINGDOM'S COALITION GOVERNMENT, FORMED IN MAY THIS YEAR, PROPOSED to eliminate the budget deficit by slashing annual public expenditures by more than £80 billion per year, there was anxiety about science funding. Would the United Kingdom follow the United States, France, and Germany (and the emerging economies of the Far East) and prioritize scientific research as part of its economic recovery and growth strategy? Or would it align with Spain and the Czech Republic, where these areas were cut? Two weeks ago, the Spending Review announced a "flat cash" science budget of £4.6 billion per year for the next 4 years (with no allowance for inflation). This gives the United Kingdom a fighting chance of preserving its science base. But major concerns remain.

Academics and the high-tech industry had argued vociferously that a severe cut in R&D spending would rattle a robust science base strengthened under the previous government and jeopardize long-term economic recovery, threatening the nation's ability to attract talent and investment from abroad. It would also send a discouraging signal to young people contemplating scientific careers. These arguments fortunately found a resonance. But science is embedded in the university system, where the prospects remain uncertain. The United Kingdom is in the throes of an upheaval in university funding, with an aim of transferring the burden "from the taxpayer to the student." Public funding for the teaching role of universities is being cut by 40%, and students will be expected to pay higher tuition fees through loans repayable out of future earnings. The Liberal Democrats (the minority partner in the Conservative-led coalition) are opposed even to current fees, capped at £3280 per year, and the cap would need to be raised to at least £7000 to compensate for the cut in public funding. The expected politically constrained compromise could prevent the universities from maintaining the infrastructure that research requires.

In the United Kingdom, research is concentrated in universities (as in the United States, but unlike France and Germany), and it is the only country outside the United States to have several universities consistently rank high in global "league tables." This strength was an undoubted factor in the United Kingdom's success in the Nobel Prize stakes this year—three winners in science, plus one in economics. The physics prize winners, Andrei Geim and Konstantin Novoselov, joined the University of Manchester in 2001, attracted to the United Kingdom by the promise of adequate funding and a supportive environment in a first-rate university. It is crucial that such people make similar choices in the future.

Total UK enrollment in full-time higher education, across all disciplines, has risen from less than 10% in the 1960s to around 40% today. But this welcome expansion has not spawned diversity in the types of undergraduate schools. In the United States, less than one-tenth of the institutions that offer bachelor-level qualifications have Ph.D. programs. The United Kingdom would benefit from likewise concentrating Ph.D.-level education into fewer institutions, accompanied by more collaboration between universities. Enhanced international collaboration is welcome too. British scientists collaborate more with the United States than with any other nation; they also benefit from the unrivaled shared facilities of Europe-wide consortia such as the European Laboratory for Particle Physics (CERN) and the European Southern Observatory.

In 2005, the U.S. National Academies published an influential report, *Rising Above the Gathering Storm*, highlighting the increasingly competitive global landscape for science. In September of this year, the update, subtitled *Approaching Category Five*, reinforced the message, remarking that: "You can't make an overweight aircraft more flightworthy by removing an engine." This message is even more vital for the United Kingdom, which must rebalance its economy away from an overdependence on the financial sector. Science and innovation are essential "engines" for long-term prosperity and confronting global challenges. We must hope that, after 4 years of straitened funding, UK science can share the fruits of the recovery that it will help to generate.

— Martin Rees

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INFECTIOUS DISEASES

Haiti's Outbreak Is Latest in Cholera's New Global Assault

Was it imported or indigenous?

That was one of the first questions cholera scientists were asking after a deadly surprise outbreak of cholera began in Haiti 2 weeks ago. Angry Haitian protesters had already decided: They held peacekeepers from Nepal—a country where the disease is endemic—responsible for bringing *Vibrio cholerae* to their already ravaged country and demanded their departure. But some scientists pointed out that although Haiti had never reported the disease before, *V. cholerae* is ubiquitous in aquatic environments; given the right circumstances, its numbers can swell dramatically and trigger an outbreak.

On Monday, the U.S. Centers for Disease Control and Prevention (CDC) rendered its verdict: DNA fingerprinting of the microbes from 13 patients had shown that it was most similar to strains from South Asia, according to a press release, suggesting that that's where the disease was imported from. (The carefully worded statement did not mention Nepal.)

For cholera experts, it was a familiar debate that often takes place when cholera pops up in a new locale. "People always assume cholera had to come from somewhere else. It's usually local," contends Rita Colwell, a veteran cholera scientist and former head of the U.S. National Science Foundation, who had put her money confidently on the environmental route.

Cholera experts agree on one thing, however: The outbreak is another coup for El Tor, the cholera biotype that emerged over half a century ago and has since slowly spread across the globe in what researchers say is cholera's seventh recorded pandemic. Recently, El Tor—"a pretty nasty beast," according to molecular biologist John Mekalanos of Harvard Medical School in Boston—has also been wildly successful in Africa, where the disease has gone from sporadic to increasingly entrenched in just the past decade. An epidemic raging in Nigeria this year, which so far has not grabbed international headlines, has already killed more than 1500 peo-

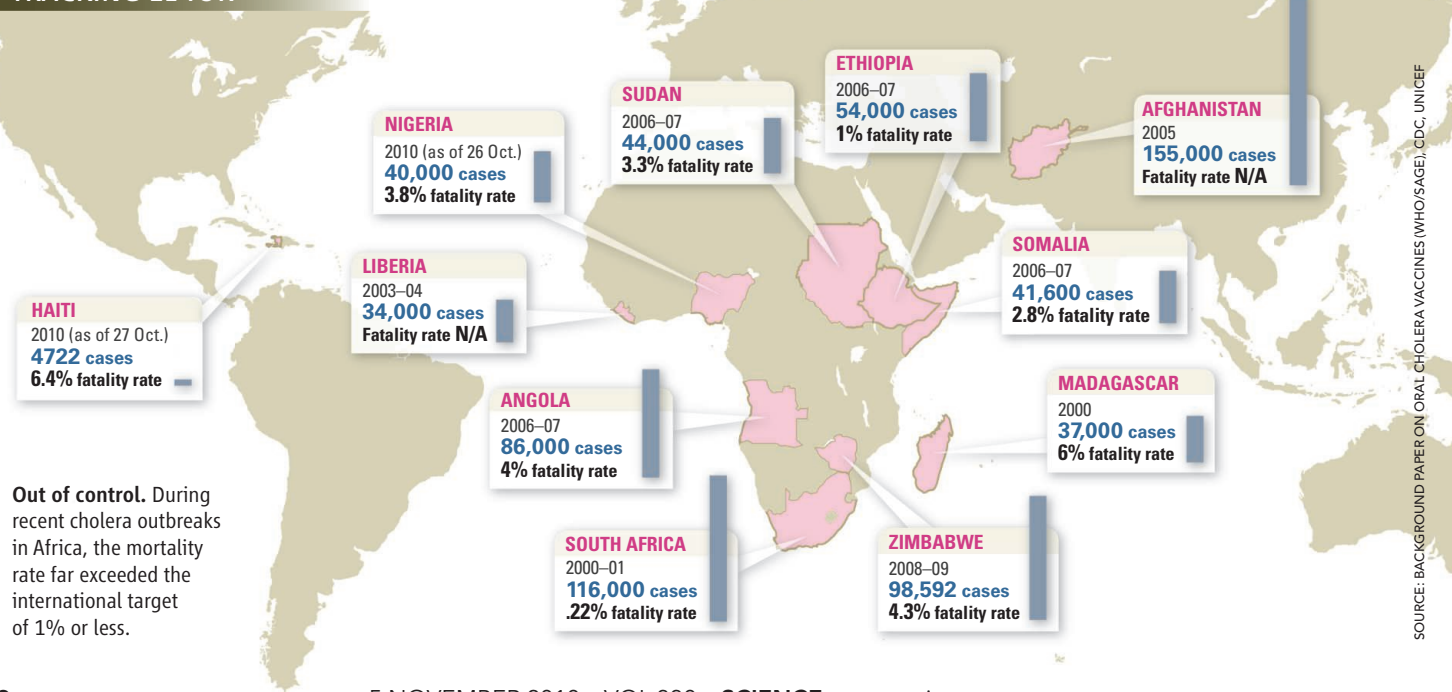
ple, and at least 550 lives have so far been lost in neighboring Cameroon. In 2008–09, Zimbabwe had a massive epidemic that killed over 4000 people.

El Tor appears to cause longer-lasting outbreaks than its predecessor, simply named "classical," and to survive longer in the environment. That could be bad news for Haiti. A preliminary study by CDC suggests the outbreak could last years and cause as many as 100,000 cases.

Few saw it coming. After the 12 January earthquake, which killed an estimated 230,000 Haitians and left more than a million homeless and living in squalid camps, health officials were worried about a panoply of diseases, including dysentery, malaria, and dengue. An international effort was launched to help the country beef up surveillance and equip the National Public Health Laboratory in Port-au-Prince for diagnostic testing.

Although cholera wasn't on the radar screen, the effort has proven useful during the outbreak, says Eric Mintz, a CDC epidemiologist specializing in waterborne diseases: The Port-au-Prince lab fingered *V. cholerae* just 4 days after the first suspected case surfaced. CDC's labs in Atlanta confirmed the diagnosis and typed the bug. (It belongs to the strain O1, like most cholera around the world, and a serotype called Ogawa.)

TRACKING EL TOR





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V. cholerae is normally present in coastal waters worldwide, even in countries where the disease is absent. It loves brackish water and can occur in rivers and lakes as well. Clinging to zooplankton—in particular, to tiny crustaceans called copepods—the bacteria can hang around indefinitely in relatively low numbers. There's no question that outbreaks can kick off when a series of environmental factors—including rising water temperatures, alkalinity, and high nutrient levels—conspire to cause zooplankton blooms, offering the cholera microbe a chance to proliferate as well. People become sick when they ingest the water and spread the disease when traces of their feces end up in other people's drinking water.

But there's debate about how often this scenario plays out. When Peru was hit by cholera in 1991, the start of a massive outbreak in Latin America that killed almost 9000, there were suspicions that the microbe had been introduced by one or more ships, presumably from Asia. But Colwell's studies suggested that the bug came from Peru's coastal waters as a result of conditions caused by El Niño. "Based on 40 years of research," Colwell says she believes something similar happened in Haiti. She is "not convinced" by the CDC statement because the agency used a technique called pulsed-field gel electrophoresis that doesn't yield a very detailed fingerprint. Colwell says she'd like to study water samples from Haiti herself but has been unable to obtain them.

But Mekalanos says the case for an environmental outbreak in Peru was never quite clinched and that Colwell and others may have dismissed the Nepalese connection in Haiti too soon. The fact that the troops were based along a tributary to the Artibonite River, where some of the first cases were found, and left home during a cholera outbreak "was a bit of a smoking gun," says Mekalanos, who is eager to do some molecular sleuthing of his own. Any infected soldiers would presumably have been asymptomatic, he says; 75% of those infected with El Tor are. (Of course, there are other potential routes through which a South Asian strain could have arrived in Haiti, he adds.)

Over the past 5 decades, El Tor has almost entirely replaced the classical biotype that scientists assume had reigned for centuries. Why it has done so is the topic of much spec-



New threat. A woman takes care of a cholera patient at a hospital in the Artibonite Province in Haiti.

ulation but few hard facts. One theory, says Mekalanos, is that the classical biotype was more adapted to reproducing in humans—and it had ample opportunity to do so until well into the 20th century. But as sanitation and drinking water started improving around the world, a strain that is hardier and better at staying alive in the environment may have gained an edge.

What's next for Haiti? Mintz and his colleagues have tried to predict the Haitian outbreak's course by extrapolating from the Latin American one, which spared the islands of the

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 Podcast interview
with author
Martin Enserink.

Caribbean. Like Haiti now, the population had little or no immunity at the time, not having seen the disease for decades. Not surprisingly, the scope of the outbreak in each country strongly correlated with basic indicators of socioeconomic development such as infant mortality, literacy, and GDP, says Mintz. In terms of development, Haiti is roughly where Bolivia was in the early 1990s, and models suggest that Haiti could face some 100,000 cases over the next couple of years, he says. "It's both extensive spread and possible persistence that we are concerned about," he says.

The key weapon is to prevent transmission where possible, with clean water, sanitation, and education, and to rush treatment to those affected. Until a few decades ago, cholera had horrific death rates of 20% or

more. The advent of so-called oral rehydration therapy—a simple solution that replenishes the body's electrolytes—in the 1970s has dramatically lowered those numbers, but it needs to be provided rapidly, as cholera can kill within a day. That can be a huge challenge in the places where cholera often strikes: poor countries with little infrastructure and a dearth of medical facilities.

Today, an unofficial international standard says that mortality during outbreaks should be 1% or lower, says Claire-Lise Chaignat, head of the Global Task Force on Cholera Control at the World Health Organization in Geneva. Some countries in Asia that experience cholera often do even better, but mortality during outbreaks rates during recent outbreaks in Africa have been several times higher (see map). Early this week, the official case fatality rate in Haiti still stood at 6.4% but was dropping.

One particular worry in Haiti is that a million or so people still live in camps, mostly in Port-au-Prince, where diseases spread easily. A 1994 outbreak among Rwandan refugees in Goma in the Democratic Republic of the Congo, in which more than 20,000 people perished, is a vivid reminder of how dangerous camps can be. So far, the disease has not reached the Haitian tent cities. Mintz, for one, says the camps are better organized than those in Goma, and people living there may actually be at an advantage compared with other Haitians because most drink chlorinated water and have a better chance at timely treatment.

—MARTIN ENSERINK

RESEARCH ETHICS

Questions From China Snag U.S. Trial Of Nerve-Rerouting Procedure

A running 5-year medical brawl in China has spilled over into Michigan, where it has delayed a clinical trial about to enroll patients. The trial, based at the William Beaumont Hospital in Royal Oak, Michigan, aims to surgically reroute the nerves of spina bifida patients to give them control of their bladder. Principal investigator Kenneth Peters confirmed last week that the U.S. National Institutes of Health (NIH)—which is funding the work—has asked for a review.

The urologist who invented the nerve-rerouting procedure, Xiao Chuan-Guo, has claimed phenomenal results in China—including an 87% success rate for 110 spina bifida patients at their 1-year follow-up visits. But the controversy surrounding his work is phenomenal, too. Earlier this year police charged Xiao, head of urology at the Union Hospital affiliated with Huazhong University of Science and Technology in Wuhan, with organizing street attacks on two of his critics. Those injured were Fang Shimin, who under the pen name Fang Zhouzi operates the *Xin Yu Si* or New Threads Web site (www.xys.org), and journalist Fang Xuanchang (no relation to Fang Shimin), who has edited magazine articles about Chinese patients who failed to benefit from Xiao's procedure.

Xiao was convicted of “causing disturbance” and sentenced to 5.5 months of detention (<http://scim.ag/doctor-sentenced-Beijing>). He has appealed the verdict. *Science* sent a request for comment to Xiao's lawyer by e-mail but did not receive a response by presstime.

Questions about the clinical trial in Michigan based on Xiao's procedure reached the U.S. Department of Health and Human Services in March, when the so-called New Threads Volunteers, a watchdog group that tracks Xiao's research, sent a letter to the Office of Research Integrity (ORI) and the Office for Human Research Protections (OHRP). The letter alleged, among other things, that “the current clinical trials in the United States are based on dubious data.”

ORI declined to take action, according to Eddie Cheng, a blogger, software engineer, and member of the Volunteers, who mailed



Under fire. Xiao Chuan-Guo's reports of success in treating spina bifida patients have been challenged by Chinese critics.

letters about Xiao's study to ORI and OHRP. Cheng says ORI wrote back in March that the allegations weren't specific and that Xiao's work in China was out of its jurisdiction. Last week, however, OHRP confirmed in an e-mail to Cheng that it had asked the funding agency to evaluate the allegations.

Xiao has many friends in the scientific community. Peters, head of urology at the Beaumont Hospital, and 30 researchers signed an open letter in support of Xiao in September urging China to “protect his human rights” and praising Xiao as “a compassionate man who is respected worldwide for his integrity and his innovative scientific contributions to society.”

Xiao developed a nerve-rerouting procedure to treat neurogenic bladder disorder in patients with spinal cord injury (SCI). Nerve crossover was first proposed by an Australian surgeon in 1907; medical literature holds a scattering of partial success stories. But Xiao's approach—which he proposed in the late 1980s—bypasses the central nervous system by grafting a lower lumbar nerve to one or two sacral nerves below the

spinal cord lesion, rerouting signals to bladder and urinary muscles. Xiao claims to have established a new pathway that can be used to initiate voluntary urination by scratching or squeezing skin on the thigh.

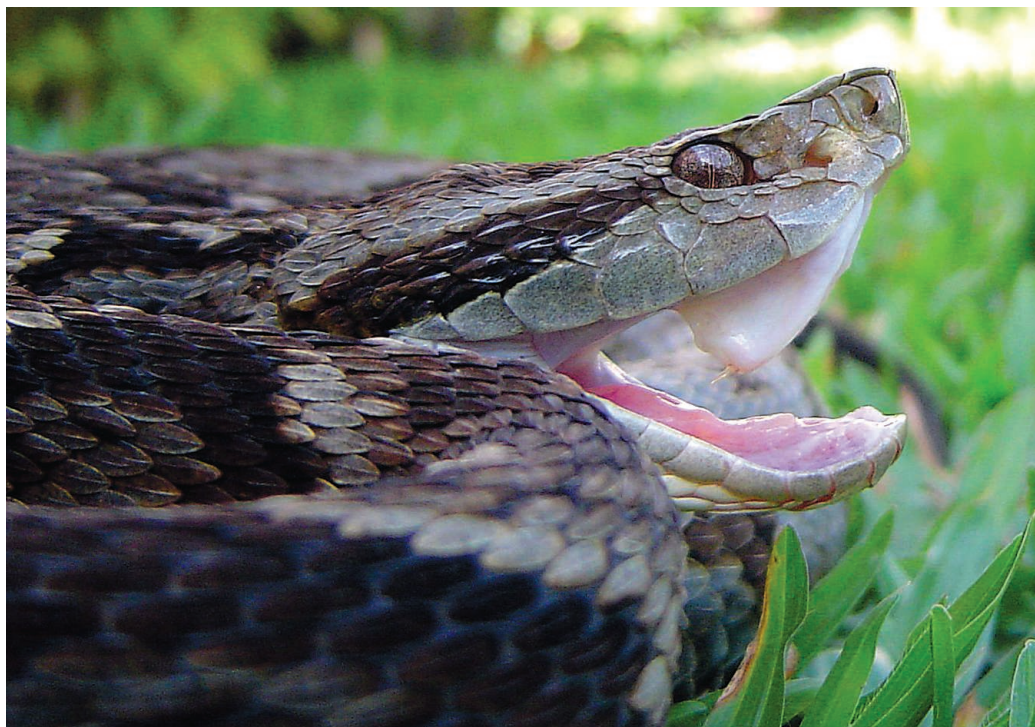
After testing the idea on rats and cats, Xiao applied for and received an NIH grant in 1994 to study dogs at the Long Island College Hospital in Brooklyn, New York. According to his own published account, Xiao began a trial of the procedure with Chinese SCI patients at a hospital affiliated with a coal mine in Henan Province in 1995 and published final results from the SCI patients in 2003 in *The Journal of Urology*. This peer-reviewed article reported that of 15 male SCI patients—all with hyperreflexic neurogenic bladder (involuntary voiding)—who had the surgery, 10 gained satisfactory bladder function, two had partial recovery, two failed, and one was lost to follow-up.

Critics see inconsistencies in the data. For example, in early reports (some in Chinese), Xiao described patients' recovery taking place between 10 and 12 months post-op, but the 2003 final report says that patients gained bladder function 12 to 18 months post-op. In addition, the depiction of all 15 patients as hyperreflexic in the 2003 report seems at odds with Xiao's previous reports, which described treating a mix of patients with hyperreflexic bladder and areflexic bladder (failure to void).

Eric Kurzrock, chief of pediatric urology at the University of California, Davis, Children's Hospital in Sacramento, California, says Xiao's study is “extremely flawed” because of “patient selection bias.” Kurzrock is particularly critical of the claimed high success rate, because it is not based on data from a randomized, controlled trial.

After treating SCI patients, Xiao began using nerve rerouting to treat bladder malfunction in children with spina bifida, whose spinal cords are generally not as damaged as those of SCI patients. The first privately funded trial at Beaumont Hospital, which took place in 2006 and 2007, included nine spina bifida patients and two SCI patients; Peters and co-authors reported preliminary results from spina bifida patients, but results on SCI patients have not been reported. The current NIH-funded trial aims to enroll about 16 spina bifida patients; the original design was not blind and had no control group. Peters says NIH has “created an oversight committee for our study. We met with them a few weeks ago and are addressing their comments. We will be submitting a revised protocol soon for their review.”

—HAO XIN



Snake oil. Brazilian pit viper venom was used to develop a blockbuster hypertension drug, but Brazil didn't profit. A new treaty gives countries a stake in the use of their resources.

CONVENTION ON BIOLOGICAL DIVERSITY

U.N. Biodiversity Summit Yields Welcome and Unexpected Progress

NAGOYA, JAPAN—Delegates, advocates, and observers were braced for the worst going into the last week of a biodiversity summit here. Instead, at a marathon closing plenary session, the parties agreed to an ambitious plan to halt the loss of biodiversity, an agreement to combat biopiracy, a scheme to increase conservation funding, and a raft of related policy statements. Not everyone got what they wanted, but most got something they could live with.

The plan to “take effective and urgent action to halt the loss of biodiversity” is a “pretty good deal” in the eyes of James Leape, director general of the Geneva-based conservation organization WWF.

The agreement to equitably share benefits resulting from research using natural resources “should help, not hinder,” academic researchers doing fieldwork while protecting the interests of developing countries, says Geoff Burton, an Australian expert on the issue.

And although funding pledges proved disappointing, the scheme to ramp up monetary support is encouraging, says Gustavo Fonseca, head of the natural resources team at the Global Environment Facility (GEF) in Washington, D.C., a multilateral entity

supporting developing world sustainable development.

These agreements, adopted at the 10th meeting of the Conference of the Parties (COP 10) to the 1992 Convention on Biological Diversity (CBD), took years of preparatory work, 2 weeks of intense negotiations here, and deliberations at a final plenary session that ran past 2 a.m. on 30 October. Delegates were relieved and proud that the meeting produced substantive results, in marked contrast to the U.N. conference on climate change in Copenhagen last December, which accomplished little.

The first of a trio of agreements many delegations wanted to treat as an all-or-nothing package addresses access to genetic resources and the sharing of benefits arising from their use. The existing CBD recognizes the sovereign rights of states over their natural resources. But developing countries had long wanted a document with more specific details, and indigenous groups have demanded recognition of their rights to resources on ancestral lands and to traditional knowledge. Advocates say there are hundreds if not thousands of examples of biopiracy: drugs, dietary supplements, and cosmetics based on traditional knowledge

of the medicinal properties of plant and animal products that have been commercialized without authorization or any benefits being returned to local communities. Brazilian delegates point to the case of the venom of *Bothrops jararaca*, a Brazilian pit viper. Locals had long known that the viper's bite thins blood so much that it seeps through vascular walls, causing a precipitous drop in blood pressure; victims often bleed to death. After a Brazilian researcher isolated the active compound, researchers in the United Kingdom and the United States elucidated the mechanism and developed a synthetic compound that mimics the

venom's effect. The company now known as Bristol-Myers Squibb put the resulting hypertension drug, Captopril, on the U.S. market in 1981. Neither Brazil nor the local community benefited from sales that ran to billions of dollars.

To resolve such issues, the convention launched formal talks to produce a protocol on access and benefit sharing—ABS, for short—in 2004. By the start of COP 10, negotiators had produced a draft document stating that those seeking to use genetic resources or traditional knowledge for research or commercialization must obtain prior informed consent from both the country and indigenous communities involved and to agree on terms to share monetary and nonmonetary benefits, including intellectual property rights.

Two particularly sticky issues weren't resolved until the last day of the meeting. Developing countries and advocates for indigenous peoples wanted the provisions to apply retroactively. In the end, retroactivity was dropped in favor of a call for a “global multilateral benefit-sharing mechanism” to address cases in which genetic resources were acquired prior to the new agreement.

Compliance was also a point of contention. The draft protocol included a long list of “checkpoints”—including patent offices, research institutions, and scientific journals—at which the proper acquisition of materials or knowledge would be verified. This, too, was simplified, leaving it up to individual coun-

tries to establish compliance systems. “It is probably the best we could do at Nagoya,” says Paulino Franco de Carvalho, head of the Brazilian delegation.

Academic researchers have worried that the protocol might hinder basic research in areas such as taxonomy. Overall, the new protocol “will assist academic researchers insofar as current obstacles faced in many countries will be removed,” says Burton, who participated in the negotiations and in the adoption of ABS legislation in Australia. He says that in the absence of an international agreement, some developing countries have made access so difficult that it can take up to 2 years for researchers to navigate the permitting process.

Under the new protocol, ratifying countries must designate a “national focal point for access and benefit sharing” to provide all necessary information on permits. It also calls for decisions to be rendered within a reasonable time. And there should be “simplified access procedures” for noncommercial research that Burton hopes will result in quick and inexpensive permitting. In turn, all parties must share nonmonetary benefits, such as joint authorship of scientific papers or at the least acknowledgment of the use of traditional knowledge, says Joji Cariño, an official of Tebtebba, a Philippine indigenous people’s advocacy group. Academic researchers will also have to track the use of any materials by third parties, as commercialization will require further negotiations with provider countries and communities.

The second of the three major all-or-nothing agreements was the adoption of a strategy to stem biodiversity loss by 2020. The plan’s 20 Aichi Targets, named after the prefecture that is home to Nagoya, include expanding protected areas to cover at least 17% of the world’s terrestrial and 10% of the total marine surface (see table). Conservationists had been pushing for 20% in total. “Studies show that’s the bottom line needed to conserve species,” says Alison Satterfield, head of science for BirdLife International in Cambridge, U.K.

WWF’s Leape says 17% “is not great” but is “a big step forward.” And targeting 10% of marine areas “is a 10-fold expansion” over the 1% currently protected, he says.

In 2002, parties to the CBD also set a number of conservation targets for 2010 and then largely missed them. In an attempt to avoid such failure, the strategic plan requires countries to describe their conservation plans and report progress to the convention. “We’re not just left with bio-aspirations,” says John Fitzgerald, policy director for the

HIGHLIGHT TARGETS OF THE CBD STRATEGIC PLAN TO ACHIEVE BY 2020:

- ▶ Eliminate subsidies harmful to biodiversity.
- ▶ Halve, or bring close to zero, the rate of loss of all natural habitats.
- ▶ Sustainably manage and harvest all fish and invertebrate stocks and aquatic plants.
- ▶ Reduce pollution to levels that are not detrimental to ecosystems and biodiversity.
- ▶ Control or eradicate prioritized invasive alien species.
- ▶ Minimize anthropogenic pressures on coral reefs.
- ▶ Conserve at least 17% of terrestrial and 10% of coastal and marine areas in protected zones.
- ▶ Prevent the extinction of known threatened species.
- ▶ Restore at least 15% of degraded ecosystems.

Society for Conservation Biology in Washington, D.C. “We have building blocks to achieve targets, and this will allow for corrective action in a timely way.”

Of course, these new efforts will take money. Japan pledged \$2 billion, but no other countries put significant new money on the table. In a new move, however, the parties did agree to increase funding according to specific percentages or amounts, with details to be worked out by the time of the COP 11 meeting, scheduled for 2012 in New Delhi, India. GEF’s Fonseca says he is “very happy” with the agreement. “This is the first time specific targets will be set; this is progress,” he says.

The ABS protocol requires ratification by at least 50 countries to go into effect. The other two agreements are effective under the CBD agreement.

In addition to approving changes in the CBD itself, delegates called for a moratorium on geoengineering schemes to avert climate change and endorsed a request to the U.N. General Assembly to create an Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services that would produce scientific assessments on biodiversity issues much as the Intergovernmental Panel on Climate Change does. “We’re quite excited about this; it’s really needed,” says Thomas Elmqvist, an ecologist at Stockholm University and a member of the Swedish delegation.

—DENNIS NORMILE

ScienceInsider

From the *Science* Policy Blog



Attorneys filed more paperwork last week in two federal courts regarding the legality of **human embryonic stem cell guidelines** set by the National Institutes of Health (NIH). One set of documents is the final word to U.S. District Judge Royce Lamberth, whose 23 August ruling briefly halted federally funded hESC research. At the same time, an appeals court has set 6 December for oral arguments addressing Lamberth’s preliminary injunction. http://scim.ag/stem_filings

A survey of some 600 intramural scientists and officials at NIH suggests that **tough new restrictions on outside consulting** have reduced by a third the number who engage in such activities. But 80% of respondents said the rules are too restrictive. The story was hotly debated in the *ScienceInsider* comments section, with one reader fearing that the crackdown could create “a backlash that harms all federally supported researchers.” http://scim.ag/NIH_consult

A new study of marine aquaculture finds that **industrial fish farms** can cause great environmental harm if there are too many of them. A new index could interest seafood buyers and policymakers evaluating how farms are regulated. http://scim.ag/fish_farm

NIH has given \$2 million grants to six scientists in a new program aimed at increasing **diversity of the scientific workforce**. One grant would create an Institute for Accessible Science, with a wet lab and a social network to help students and scientists with disabilities. http://scim.ag/sci_diversity

Senator Chuck Grassley (R-IA) is concerned about **outside groups paying for travel by intramural researchers** at the National Cancer Institute. Staffers say the trips, often to other countries, are an important way to interact with the international science community. <http://scim.ag/sponsored-travel>

For 2010 election coverage and more science policy news, visit <http://news.sciencemag.org/scienceinsider>.



On shaky ground. Debris flows this summer devastated Yingxiu (left) and Qingping.

EARTHQUAKE RECOVERY

Slew of Landslides Unmask Hidden Geological Hazards

CHENGDU, CHINA—Just before midnight on 7 August, heavy rains in the mountains north of Zhouqu, in western China's Gansu Province, unleashed a torrent of mud, boulders, and rubble on the slumbering town. Barreling in at about 10 meters per second, the debris flow smashed approximately 5500 houses, leaving 1765 people dead or missing.

Ma Dongtao had suspected that Zhouqu was a tragedy waiting to happen—and he warned about the risks about a decade ago. In the summers of 1996 and 1997, Ma, a geotechnical expert now at the Institute of Mountain Hazards and Environment (IMHE) of the Chinese Academy of Sciences in Chengdu, and a colleague assessed hazards in the Zhouqu area. Like many settlements in mountainous regions, Zhouqu is built on an alluvial fan from earlier debris flows. The August disaster was the 12th damage-causing event since 1823. The researchers estimated that some 25 million cubic meters of loose rock and sediment had accumulated in two gullies north of town. Human activities like quarrying contributed to the precarious mass, but most of it, Ma believes, was deposited by landslides and rock falls after a big earthquake in 1879. The findings suggested that debris flows could strike “more than 100 years after a major shake,” Ma says.

If he is right, many river valleys in western China harbor hidden hazards. According to Tang Chuan, a geologist at the State Key Laboratory of Geohazard Prevention and Geoenvironment Protection (SKLGP) at Chengdu University of Technology, some 60,000 landslides occurred in valleys and gullies during and shortly after the magnitude-7.9 Wenchuan earthquake that struck in May 2008.

During this year's rainy season now drawing to a close, downpours triggered more than 1000 debris flows across China, mostly in western Sichuan. So far, the number of debris flows in 2010 is more than double the total for

2006, a bumper year for geological disasters. Experts blame the Wenchuan quake, and they warn that such hazards pose long-term challenges to earthquake recovery.

Within a week of the Zhouqu disaster, hundreds of debris flows to the south in Sichuan Province blocked roads and overran at least 11 settlements for people displaced by the Wenchuan earthquake. On the night of 13 August, a debris flow near the epicenter dumped about 700,000 cubic meters of sediment into the Min River, which overran its banks and flooded resettlement homes in Yingxiu.

The same night, a giant debris flow inundated Qingping, site of the second biggest landslide triggered by the Wenchuan earthquake. The flow's runout, about 3 million cubic meters of sediment, was double that of Zhouqu, according to a survey by SKLGP geologist Yu Bin. But the Zhouqu disaster had put Qingping on high alert. When rains turned heavy at night, the town was evacuated about 2 hours before the debris flow struck. The toll—14 dead or missing—could have been far higher. But the threat to Qingping continues. Two more debris flows pummeled the town on 19 August and 18 September. The total runout of the three flows was a small fraction of the 50 million cubic meters of boulders, rocks, gravel, and soil dumped into the gully by the quake-triggered landslide, says Yu.

The third rainy season since the Wenchuan earthquake has been a wake-up call. According to Tang, experts have underestimated the potential severity of postearthquake secondary disasters. In the aftermath of the 2008 earthquake, the Sichuan government commissioned an SKLGP team to assess future hazards to help guide reconstruction. The team's report that October forecast an elevated risk of landslides and debris flows in the earthquake zone for 3 to

5 years after the quake. Tang, however, thinks the risk will remain high for at least a decade.

Indeed, this season's disasters could be a harbinger of future woes. So far, debris flows in the 2008 quake zone have occurred in small catchments with drainage areas of less than 10 square kilometers. It takes longer for sediments to be transported out of larger catchments; for that reason, disasters in drainage basins with areas bigger than 10 kilometers could strike 20 or more years after a tremor, says IMHE's Xie Hong, who has studied regional geological hazards for more than 2 decades. “We should be worried about dormant debris flow gullies in large catchments in the earthquake zone,” says Xie. Because sediment builds up gradually in large catchment areas and it takes heavier rains to sweep it out, “people have forgotten about past disasters and have built houses and factories in these gullies,” says IMHE geotechnical engineer Wang Quancai.

Qingping's leaders made the right decision by evacuating—but they also got lucky. It's hard to accurately predict geological hazards, Xie says. Part of the challenge is that debris flows are primarily triggered by localized torrential rains that are difficult to forecast.

The surest way to safeguard lives and property is to avoid building near debris flow paths, says Xie. Field surveys can look for telltale signs of dangerous buildups. After the 1999 Chi-Chi earthquake in Taiwan, debris flows in the affected area were more frequent and triggered by smaller amounts of rainfall, says Tang. He and Yu suspect that this phenomenon—threshold lowering—is occurring in the area affected by the Wenchuan quake. They are racing to gather precipitation data and survey as many disaster sites as possible to estimate runouts and flow rates before next season's debris flows bury this year's—both compounding and further cloaking the risk.

—HAO XIN

From *Science's* Online Daily News Site

Did 'Snowball Earths' Trigger Animal Evolution?

Researchers have noted that two globe-girdling ice ages roughly coincided with the appearance of the earliest animals in the fossil record. Now biogeochemist Noah Planavsky of Woods Hole Oceanographic Institution in Massachusetts and colleagues propose in *Nature* that "snowball Earth" glaciations could have spurred evolution by increasing oxygen in the air and oceans.

During a snowball-Earth episode, the team argues, the ice sheets ground up continental rock, releasing phosphorus. This key nutrient for marine plants then washed into the oceans when the glaciers retreated, fertilizing algal blooms that could drive a surge in the production of organic matter. And the added organic matter that settled into the mud on the ocean bottom would leave additional oxygen behind, eventually boosting atmospheric and oceanic oxygen.

To check how the oceans' phosphorus content varied over time, the team measured phosphorus in minerals formed in ancient oceans. During the past 3 billion years, phosphorus abundance varied little, except for a surge lasting from about 750 million years ago to about 635 million years ago, at the same time as two snowball-Earth episodes.

Other researchers call the link a fascinating idea but say it needs more support from the geologic record. <http://scim.ag/ice-O2>

Hellish 'Super-Earths' Abound

Bad news for E.T.: Astronomers predict that many of the galaxy's planets orbit so close to their parent stars that their surfaces are seas of molten lava—hardly ideal conditions for life.

Kevin Schlaufman, a graduate student at the University of California, Santa Cruz, and

NEW MONKEY SPECIES ALLERGIC TO RAIN?

Scientists have discovered a new monkey species in the mountain forests of Burma. But with only an estimated 260 to 330 individuals alive, *Rhinopithecus strykeri* is already critically endangered, Thomas Geissmann of the University Zurich, Irchel, in Switzerland and colleagues report in the *American Journal of Primatology*. Locals call the creature "mey nwoah" or "myuk na tok te," meaning "monkey with an upturned nose." Popular legend says the monkeys' uplifted nostrils make them sneeze when it rains, so they endure downpours by tucking their heads between their knees. The animals are so rare that primatologists have yet to glimpse one alive. Instead, they relied on information from hunters and carcasses to estimate their numbers and create the image above using Photoshop. Although not a key game species, the monkeys often get caught in bear traps, and the influx of Chinese logging companies is further jeopardizing their habitat, the researchers say. <http://scim.ag/new-monkey>



colleagues used computer models to simulate a theoretical extrasolar planet population. A large number of these alien worlds were hot super-Earths, rocky planets up to 10 times the mass of Earth that orbit their host stars in 24 hours or less, the team reports in a paper to be published

in *The Astrophysical Journal Letters*.

Although such planets start off farther away from their stars than Earth is from the sun, their stars slowly pull them in over about 100,000 years, ripping them apart and eventually vaporizing them. Schlaufman notes that life on these scorching planets is totally out of the question.

NASA's Kepler mission (pictured) to find Earth-like worlds is expected to announce the discovery of a slew of such hot super-Earths by early next year. <http://scim.ag/hot-earths>

Read the full postings, comments, and more at <http://news.sciencemag.org/sciencenow>.



Love Bugs

Bacteria that inhabit fruit flies help sway their choice of partners, according to a new study.

Recreating a 1980s experiment, microbiologist Eugene Rosenberg of Tel Aviv University in Israel and colleagues split one population of flies into two and fed them different diets. When combined after many generations, female flies preferred to mate with males raised on the same diet, a finding that had perplexed previous experimenters.

Rosenberg and his colleagues thought this was because changing the flies' diet changed their bacteria. Sure enough, when the flies were treated with antibiotics before being placed together, the female flies were no longer choosy. The two groups of flies also had different levels of four sex hormones that signal when the flies are ready to mate, the team reports in the *Proceedings of the National Academy of Sciences*. The bacteria may even be driving speciation by causing individuals to mate selectively with each other over so many generations that they eventually become distinct from their brethren. <http://scim.ag/love-bugs>



CHINA

Supercomputer Leaves Competition—And Users—in the Dust

FUZHOU, CHINA—China's new supercomputer, Tianhe-1A, is also the world's fastest, topping by 47% the current titleholder, the Jaguar XT5 system at Oak Ridge National Laboratory in Tennessee. But while Chinese officials are hailing the 2.5-petaflops supercomputer as an example of indigenous innovation, some Chinese researchers are troubled by another fact: The number of their colleagues able to tap even a thimbleful of the machine's power is surely minuscule.

Tianhe-1A was developed by the Central Military Commission's National University of Defense Technology (NUDT) in Changsha and housed at the National Center for Supercomputing in Tianjin, which is classified as a secret facility. Despite its pedigree, the machine is expected to focus on civilian applications, including weather forecasting, animation, and modeling of petroleum reserves, according to Liu Guangming, director of the supercomputing center.

It may be years, however, before Chinese software catches up with the hardware. It's a "big embarrassment" for supercomputer developers in China that their powerful machines have few users, says Xu Rongsheng, a computational physicist

turned network security expert in Beijing. "Few Chinese researchers know how to model a problem mathematically," says Xu.

Under the hood of the supercomputer, housed in 120 refrigerator-sized cabinets, are 14,336 Intel Xeon CPUs and 7168 Nvidia Fermi graphics boards. NUDT engineers devised a novel interconnect—twice as fast as the U.S. standard—to take advantage of the number-crunching speed of graphics chips and the capacity of CPUs to solve complex equations. Developers have also swapped in some of China's own Feiteng-100 CPUs for Intel chips.

"The performance has proved that our CPU is very powerful," China's science minister, Wan Gang, said here this week at the annual meeting of the Chinese Association for Science and Technology. "It's a substantial enhancement of the Tianhe-1 computer that was number seven on the [most recent] Top 500 list," says Jack Dongarra, a computer scientist at the Uni-

versity of Tennessee, Knoxville, whose new listing of the fastest computers in the world comes out on 15 November.

Applications, however, are where the rubber will meet the road. "Nurturing users for supercomputers is like a restaurant," Liu told Xinhua. "It not only needs to cater to customers with different tastes but also needs to have large-account customers to fill the restaurant."

For now, most supercomputer users in China are ordering off the kids' menu. Only 1% of the applications on China's previous speed champ, the Dawning 5000A at the Shanghai Supercomputer Center, use more than 160 of the machine's 30,720 cores. For comparison, 18% of the applications running on Oak Ridge's Jaguar XT5 use 45,000



New leader. Software engineers work on China's Tianhe-1A supercomputer, which is the world's fastest.

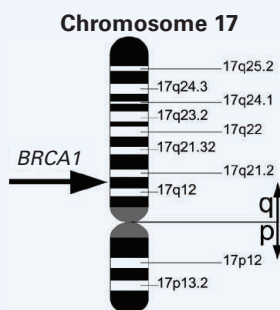
PATENT POLICY

Amicus Brief Unfriendly to Gene Patents

The U.S. Justice Department waded into a battle last week over whether genes can be patented, surprising many with a sweeping new proposal. It argued in an amicus brief filed with the U.S. Court of Appeals that "genomic DNA that has merely been isolated from the body without further alteration or manipulation" should not be eligible for patenting. DNA of this type should be treated as a "product of nature," not an invention, the brief says.

Experts disagree on the likely impact of this principle—if adopted. The Biotechnology Industry Organization

Locus of dispute. Patenting this gene was a mistake, says the U.S. government.



(BIO), a Washington, D.C., lobby, says the new stance could undercut many biotech ventures, particularly in agriculture. In a statement, BIO President and CEO James Greenwood said limiting gene patents would be at odds with "more than 2 decades" of U.S. national policy. Greenwood warned of dire consequences: If adopted, the change "would undermine U.S. global leadership and investment in the life sciences, harm U.S. economic growth and competitiveness at home and abroad, and be counterproductive to the Administration's own initiatives to fight cancer, develop renewable sources of energy," and other goals.

But the new position really is "not all that far-reaching,

despite the rhetoric," says Robert Cook-Deegan, an expert on genetics and patents at Duke University's Center for Genome Ethics, Law & Policy in Durham, North Carolina. Companies with early gene patents that want to maintain exclusive control of DNA might be affected, he says, but not younger ones that aim to develop products based on whole-genome analysis, where thousands of genes must be used.

One company sits squarely in the crosshairs: Myriad Genetics in Salt Lake City, Utah, which owns patents on the breast and ovarian cancer genes *BRCA1* and *BRCA2*. In March, a New York federal district court ruled that some of Myriad's key *BRCA* patents were invalid and should never have been granted (*Science*, 9 April, p. 153). The judge in that case agreed with the Public Patent Foundation and the American Civil Liberties Union in New York City, which wanted the patents thrown out on grounds that "isolated DNA" is not an invention. Myriad appealed.

CREDITS (TOP TO BOTTOM): GENO ZHOU/EPA/LANDOV; ARMIN KÜBELBECK/WIKIMEDIA COMMONS

to 90,000 of the machine's 150,162 cores, according to a presentation at last year's announcement of China's top 100 fastest computers. "A supercomputer without software is like a wild horse without a harness," says Zhang Yunquan, a parallel computing researcher at the Institute of Software of the Chinese Academy of Sciences in Beijing. "Its horsepower is wasted."

The problem, in part, is cost. Most industrial applications on Chinese supercomputers use commercial software purchased from the United States and other countries, Zhang says. But because the price is proportional to the number of CPUs supported by the software, few Chinese users can afford licenses that support large numbers of CPUs.

Wan told *Science* that he would like to see China develop its own applications in areas such as climate modeling and biotechnology. That would require a considerable cash infusion in the 12th 5-year plan, set to begin next year. Software now gets only a penny of every dollar spent on hardware development, Zhang says.

It may take months for Tianhe-1A to close in on its theoretical peak speed of 4.7 petaflops. And by then it almost surely will have relinquished its top rank. According to Don-garra, five supercomputers in the 10-petaflops class are being built in Japan and the United States, home to more than half the supercomputers on the top 500 list.

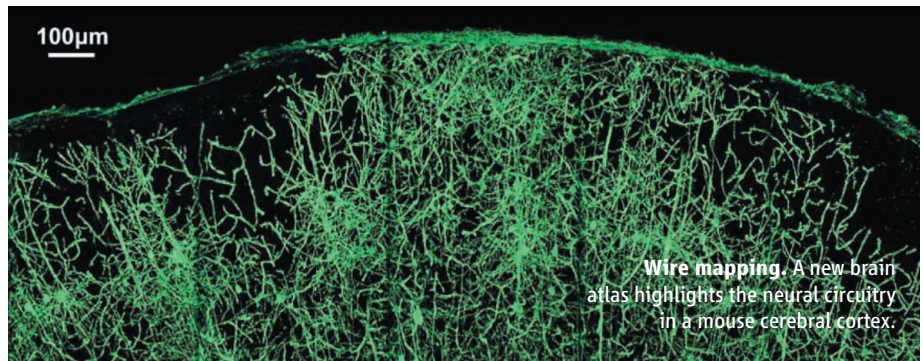
—RICHARD STONE AND HAO XIN

In its amicus brief, Justice agreed in part with the lower court judge. "The chemical structure of native human genes is a product of nature," Justice said, "and it is no less a product of nature when that structure is 'isolated' from its natural environment than are cotton fibers that have been separated from cotton seeds or coal that has been extracted from the earth."

But Justice's brief also seeks to limit the scope of the ruling handed down by the New York court, arguing that some DNA patents—such as those covering complementary DNA, vectors, and recombinant plasmids—should continue to be allowed.

It is not clear who directed that this brief be prepared; some in the biotech community speculate that it was initiated by policymakers at the White House. Nor is it clear whether the brief presages an actual change in patent policy. When asked about this, a Justice Department spokesperson said only, "the brief speaks for itself."

—ELIOT MARSHALL



NEUROSCIENCE

China's Brain Mappers Zoom In on Neural Connections

Anyone in business, and even just fans of *The Apprentice*, knows that connections matter. Neuroscientists understand that, too, which is why a research team in China has gone to great lengths to create the most detailed three-dimensional map yet of all the connections between the neurons in a complete mouse brain. The project, unveiled this week online in *Science* (www.sciencemag.org/cgi/content/abstract/science.1191776), hasn't revealed any major surprises so far, but its data and the new automated instrument that produced the brain atlas provide an important foundation for future studies, researchers say.

Mapping all the connections between neurons is crucial to understanding how the brain functions, or malfunctions, explains neuroscientist Mihail Bota of the University of Southern California in Los Angeles. Trying to figure out the brain without looking at its wiring, he says, is like trying to fix a car with all the right components but no idea how they're connected: "You don't know the input, you don't know the output, you don't know how the signal is processed."

The new brain atlas project resembles several recent efforts, though the previous ones have not captured the same kind of detail as the China effort. Last year, a team of California researchers began slicing up the brain of psychology's most famous amnesiac, Henry Gustav Molaison, to figure out what exactly messed up his long-term memory (*Science*, 26 June 2009, p. 1634). And in 2006, the Allen Institute for Brain Science in Seattle, Washington, spent \$41 million on its own 3D mouse brain map, which documents gene expression at a cellular resolution in an online database available to neuroscientists.

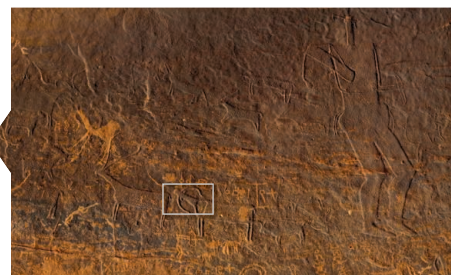
But neither of those projects imaged a whole brain at a resolution showing the axons and dendrites connecting all the nerve

cells. To accomplish this challenging task, the researchers, led by Li Anan of the Britton Chance Center for Biomedical Photonics in Wuhan, China, used a traditional slice-and-scan technique but in a newly automated fashion. After removing the centimeter-size brain from a mouse, they soaked the organ for 6 months in a cellular stain, then dried and baked it. Next, they placed the brain on a chopping block in the micro-optical sectioning tomography machine, a new instrument the team developed that incorporates a diamond knife that can slice tissue into wafer-thin strips micrometers thick. A light microscope and image recorder immediately captures a picture of each brain strip before the next one is cut. The instrument worked 10 days nonstop to collect the data—eight terabytes in all—for the mouse brain atlas.

Documenting normal neuronal connections may be important because many of the brain's more complicated maladies, such as autism or schizophrenia, may be rooted in circuit problems, according to neuroscientist Jason Bohland of Boston University. There's a growing feeling among neuroscientists, he says, that social or linguistic disorders could be caused by disruptions in the pathways between nerve cells—and in order to figure out what's going on at that level, scientists need more detailed maps. "You don't have complex behavior emerging at the level of brain regions. You have it emerging at the level of circuits," he says.

The new images offer a broad, comprehensive outlook on the brain, one more focused on the brain's architecture than its biology, says Kelly Overly, a neuroscientist at the Allen Institute. Still, it could be a valuable comparison tool for more specific, targeted studies. "There's so many things you could do with this sort of data," says Bohland.

—KRISTEN MINOGUE



Panning for Science

A new technology for creating and viewing stunningly high-resolution panoramic images is becoming a powerful research tool

WHEN NASA'S TWIN MARS ROVERS BEGAN sending detailed pictures to Earth in January 2004, Randy Sargent, a computer scientist working on visualizations of those images, was enthralled by the sense of actually exploring martian terrain. Onboard each rover, a camera known as the Pancam swiveled and tilted on command from NASA scientists. Sargent and his colleagues combined each exposure into a stunning digital panorama of the Red Planet's landscape. Scientists at the Jet Propulsion Laboratory in Pasadena, California, could interact with the images on their computer screens, zoom in on fine details, hypothesize about what they were seeing, and pick the rovers' next destinations. "The pan had so much resolution, it felt like peering through a little hole in the wall into another world," recalls Sargent's manager, robotics group leader Illah Nourbakhsh at NASA Ames Research Center in Moffett Field, California, who was then on sabbatical from Carnegie Mellon University (CMU) in Pittsburgh, Pennsylvania. "What stunned us was this feeling of presence, which a simple picture that is not interactive doesn't give you."

That experience led directly to a technology that has become a powerful tool for teaching and public engagement with science and the natural world. Scientists are also using it for projects as diverse as analyzing Middle Eastern petroglyphs, monitoring an urban

forest, archiving a museum insect collection, studying a collapsed honey bee colony, keeping tabs on glaciers, examining erosion in a jaguar reserve, and viewing Galápagos fish clustered into a bait ball.

Soon after the martian panorama renderings, Nourbakhsh challenged his team to think creatively about "blue sky" projects they could tackle. Aware of the intense reverence astronauts felt as they gazed at Earth from space, Sargent proposed bringing that kind of experience down to Earth by building affordable equipment anybody could use to create explorable images. Nourbakhsh immediately recognized the idea's potential for changing the relationship between viewer and image. "An explorable image is a disruptive shift away from the static image you just glance at, because now you have the power of exploration," he says. "That sets people up with a different mindset because *they* decide where to zoom, where to go, what structures and details to see. And it's not virtual, it's not a video game. It's real."

Sargent developed a prototype for what is now the GigaPan system. Users punch numbers into a keypad on a robotic mount for a digital camera, specifying how expansive they want their panorama to be. A microprocessor calculates the size and number of exposures needed for the pan and moves the camera accordingly. A small robotic finger pushes the shutter button for each exposure. These are

Rock art. A GigaPan of a remote outcrop in Saudi Arabia helps see and date drawings chipped through the "desert patina" on the rock face.

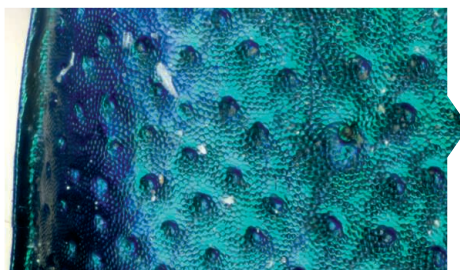
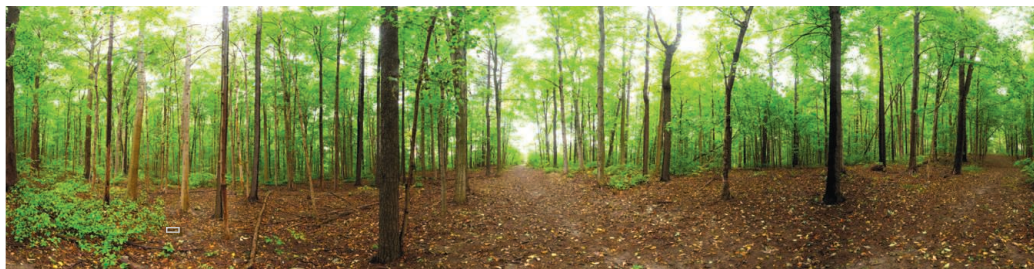
stitched together to form a panorama with a resolution 1000 times that of HDTV. The largest GigaPan has 100 gigapixels.

The final image contains more data than most personal computers can handle, so Nourbakhsh and his team developed a massive server system and Web site, www.gigapan.org, for storing and accessing GigaPans. When viewers zoom in on an area of an image, they seem to fly into the image itself. The result is an immersive, interactive experience that can reveal surprising details—an ant on a leaf in a forest, or a hummingbird sipping nectar from a flower in a backyard. It's like viewing nature through a huge magnifying glass.

Sargent, who now has a joint appointment at NASA Ames and CMU, and Nourbakhsh, head of CMU's CREATE Lab, founded GigaPan Systems LLC in 2008 to manufacture and sell the systems at close to cost (\$299 to \$895). They are continuing to develop technology for creating and viewing high-resolution scans as co-principal investigators of the Global Connection Project, a joint development between NASA Ames, the CREATE lab, Google Inc., and the National Geographic Society.

Gigapixel science

GigaPans have captured the public's imagination. Five thousand systems have been bought, and today the site hosts 40,000 public panoramas that draw 20 million visitors a year; another 20,000 are in private areas of the site while contributors work on them. Sargent and Nourbakhsh began training scientists to shoot GigaPans in 2008, and some 120 investigators



are now using the system in their research. In mid-November, scientists will share findings at the Fine International Conference on Gigapixel Imaging for Science, hosted by CMU and the CREATE lab.

Paleontologist K. Christopher Beard and archaeologist Sandra Olsen of the Carnegie Museum of Natural History in Pittsburgh and photographer Richard T. Bryant trained their GigaPan on remote Saudi Arabian petroglyphs, including the Eagle's Nest in Juba. The engravings date back to the Holocene Wet Phase (9000 B.C.E.–5000 B.C.E.) when the environment resembled that of today's African savanna, and lions, gazelles, cheetahs, wild asses, and hyenas roamed the land. Camel drawings accompanied by writing appeared in 1500 B.C.E., followed by images of horses and chariots. "You can see grains of sand, details of grooves and peckings, and the relationships between the images, which is important for dating," marvels Olsen, who runs the museum's anthropology section. The technology reduces the time spent on expensive on-site research. "We can be there briefly producing GigaPans, take that data home, and study images at our leisure on a wide-screen computer," Olsen says.

The technology also lets Olsen share data easily with colleagues and peer reviewers. "This is the closest to being there," says Olsen. Such data sharing lets colleagues perform the same kind of analysis she does, and below her panorama they can post details they found and annotate them.

M. Alex Smith, a molecular ecologist at the University of Guelph in Canada, is deploying the technology closer to home. He is using GigaPans to monitor the "Dairy Bush," an

urban forest that has been part of the Guelph campus since 1830. The 8.5-hectare wood lot contains rare and listed species, but this living laboratory for ecology students has degraded as its surroundings changed from farmland to condos and shops, and invasive species gained a foothold. To monitor such pressures, Smith abandoned the rough, "Victorian method of pencil and paper" for the GigaPan's precision. Since August 2009 he has taken weekly shots from the same location, which enabled him to discover phenomena and return with questions he had not known to ask. "There's a chokecherry on the lower left quarter of the pan full of insects from spring to fall that I wasn't seeing," he says, "and assassin bugs and parasitic wasps I wasn't aware of." Users worldwide have discerned, grabbed, and sent shots of snails, caterpillars, and trash.

Smith has also produced a time-lapse video of his series of pans, now on YouTube. The video helped him study the formation of the Dairy Bush tree canopy, which is critical to quantifying a forest's response to climate change. Time-lapse video is not zoomable now, but at the November conference Sargent and Nourbakhsh will unveil a viewer that will let users zoom and move back and forth in time.

For John Rawlins, curator of invertebrate zoology at the Carnegie Museum of Natural History, the GigaPan is an opportunity to archive and share a 17-million-bug collection. The advantage of "pixel traveling" is "scary in a way," Rawlins says, because the staggering sharpness reveals minutiae—spinules (thorny spikes) on legs or the microsculpture of forewings, for example—that can easily be missed while examining a bug under a micro-

Fine details. Snails in an urban forest and the texture of a beetle's carapace show the power of GigaPans to home in on details.

scope. Rawlins forecasts that such detailed and accessible archives will allow researchers to identify insects rapidly using automated image analysis and pattern searching.

With that goal in mind, Rawlins is collaborating with Gene Cooper, president of Four Chambers Studio in Vallejo, California, and CMU to develop a microGigaPan using an optical microscope. One effort involves imaging a Powdermill butterfly from the 1950s. Rather than compare two side-by-side photographs of its front and back, this system makes possible simultaneous inspection through pixel layers of varying transparency, so the viewer can evaluate wing markings as if passing through the insect. And the next step beyond microGigaPans is coming soon: nanoGigaPans, with as high as 8000 times magnification (0.01 gigapixels). NASA Ames's Jay Longson and Rich Gibson have adapted scanning electron microscopes to produce extreme close-up pans of insects, cells, and seeds.

There are drawbacks to the technology. It can take hours to shoot sections of a pan and stitch them together, for example, and sometimes the stitching software has trouble matching uniform patches, like sky. And Nourbakhsh is concerned that his team has created yet another technology that enables people to spend all their time exploring on their computers instead of experiencing the world firsthand. But from martian vistas to cellular landscapes, GigaPans are putting science in the picture in interesting new ways. Some day a picture may well be worth 1000 gigapixels.

—KAREN A. FRENKEL

Karen A. Frenkel is a science writer in New York City.

Online

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HYDROLOGY

Out of the Mist

Water-starved communities are betting that a fog-harvesting forest will one day quench their thirst



Netting the fog. Large plastic nets suspended on top of a hill trap moisture from the sea air to provide water in coastal Lima, where it rarely rains.

LIMA—On a sand dune on the outskirts of this city, residents of a shantytown are attempting to grow a forest. This coastline is one of the driest regions on Earth—Lima receives less than 1.5 centimeters of rain per year. Nevertheless, the forest plan is not as crazy as it sounds, as these Incan hills were wooded until Europeans cut the trees for lumber in the 1600s.

Javier Torres Luna is hoping that reforesting the dunes above his hastily constructed plywood home will provide a long-term solution to an urgent problem: There is no water in his community for drinking, washing, or sanitation. And there is certainly none for forestry irrigation.

But change is in the air. Literally.

It's not that there's no water in Lima; it's just that there's no rain. From May through November, the chilly Humboldt ocean current cools the water-laden air coming from the Pacific, preventing rain along the coast. Instead, this thermal inversion blankets the city in a thick, gray fog.

Over the past couple of years, Luna and his neighbors have erected a series of 4-meter-high nets at the top of the dune to capture precious drops from the wet air. In as few as 4 years, Luna's irrigated saplings will themselves trap the fog, creating a microclimate that should yield a self-sustaining runoff.

Fog harvesting and other experiments,

such as painting mountains (see p. 751), are how Luna and other Peruvians are trying to adapt to a heightening water crisis. Peru is losing its glaciers, a key source of water for the country, and the government is struggling to come up with solutions, particularly for Lima. "Fog nets are an extremely useful idea," says Elizabeth Silvestre, scientific director of the National Meteorological and Hydrological Service in Lima. "As water shortages become more of a pressing issue, we are going to have to expand adaptive techniques like this."

A thirsty city

The world's largest desert city after Cairo, Lima is entirely dependent for its water and its electricity on the seasonally erratic, drought-prone Rimac River. Two-thirds of the glacier at the Rimac's headwaters has disappeared, decreasing the river's glacier-contributed volume by 90% in the past 40 years. The little—highly contaminated—water that remains is primarily from rainwater.

Unfortunately, because of climate change, the glaciers are only getting smaller. Not only are warmer temperatures melting glaciers, but rain that used to fall as snow is also washing them away. In just 30 years, Peru's total glacier-covered area has shrunk from 2000 to 1500 square kilometers. That represents an estimated 7 billion cubic meters of water—the equivalent of 10 years of water consumption

in Lima. To make matters worse, the lakes at the base of the glaciers, on which thousands of rural people depend, are disappearing as well.

Water shortages and drought have driven many people, such as Luna, from the countryside to the cities. More than 80% of Peru's population now lives on the country's desert coastal strip. Well over half of them—9 million—call Lima home. This migration is putting even greater pressure on the city's dwindling water resources.

Part of the problem is that the state agency charged with managing Lima's water supply, Sedapal, is not doing a great job, losing by its own admission some 40% of the city's 220-million-cubic-meters-per-year supply through leakages and theft. Even in the wealthier parts of downtown Lima, businesses and residences periodically have nothing flowing through their taps.

But the problem is far worse in the 1800 slum communities that are home to some 2 million migrants. There, it can take decades to be hooked up to the city's supply, says Abel Cruz, director of Peruvians Without Water, an action group started by Cruz in reaction to the poorest Peruvians having to pay the most for water. Lacking a municipal source, they must hire private water trucks, paying 10 times what downtown Lima residents pay for city water.

"And because these water companies are private and not monitored, they supply cheap, contaminated water that makes us sick," says Cruz, who lives in a community neighboring Luna's. The slums have a high incidence of dysentery and other waterborne diseases.

Out of thin air

Luna and his neighbors have given up waiting for the city's help. With assistance from the German nonprofit organization Alimón, the community has built reservoirs and tanks and constructed 8-by-4-meter plastic nets, supported by steel cables secured to poles, on the top of the dune to trap the precious fog. Fog-carrying wind from the ocean collides with the nets, and all the condensing water sounds like a gushing fountain. In the sunny season, January to April, weeks can go by without the nets collecting a drop, but from May through November, the fog is a thick, daily occurrence. The record catch per net is 590 liters in a single day. "We had no idea it would work so well," Luna says.

Water courses down the nets where it is captured in gutters and stored in tanks. It falls by gravity, irrigating tree saplings, and then continues through a sand filter after which it can be used by the community. The fog water collected is more than sufficient

for the tree nursery but not enough for everyone's daily needs—currently a meager 10 to 15 liters per person. For that they must wait until their cloud forest has grown enough to make the nets redundant.

Fog collecting is not a new idea: Indigenous cultures from Europe to Africa have exploited the natural fog-harvesting properties of trees to catch their water. In the Spanish Canary Islands, for example, people used to construct funnels at the base of trees to collect the fog runoff.

Alimón biologist Kai Tiedemann spent a decade researching the fog-harvesting properties of various Canary Island trees. “From walking through a forest, it’s possible to see that some trees harvest fog—they are dripping—whereas others are dry,” Tiedemann says. He tested several types of trees and bushes, hanging the branches from a line suspended in the coastal fog and measuring the volume of water produced by each. “The ones with needlelike [rather than broad] leaves were the best fog harvesters,” and those with leaves oriented vertically rather than horizontally were the best, he explains.

Certain trees are highly adapted to harvest fog water—some, like the Californian redwood trees, satisfy the majority of their water needs in this way. The trees create a physical barrier that intercepts and precipitates fog that would otherwise rise and dissipate in the warm air. In doing so, the trees create a localized water cycle: The fog water collected on leaves drips down and nourishes grasses, shrubs, and other plants that in turn trap their own water. All this dripping water sinks into the ground, filling wells and giving rise to small streams that people can use.

The technique works. In the 1800s, British naturalist Charles Darwin helped to make the dry, volcanic Ascension Island habitable for British troops stationed there by foresting a hill with seedlings brought from botanic gardens in London. In 20 years, there was enough water to grow food for hundreds of



Urban forestry. Residents of this slum settlement are hoping to use fog water to reforest the hill above their homes.

troops. “The once-barren hill is now known as ‘Green Mountain,’” says ecologist David Wilkinson of Liverpool John Moores University in the United Kingdom, who is an expert on the island’s ecosystem. “This experiment shows that such an ecosystem that would normally take millions of years to develop can be created in a matter of decades.”

Tiedemann and his partner Anne Lummerich decided to try this approach in the bone-dry outskirts of Lima. They selected a native species, *Caesalpinia spinosa* (“tara” in Spanish), for planting. It was the second-best collector among four they tested, but it is commercially valuable, so it offers two benefits: to harvest fog water and to produce fruit that could generate new income for the community. Tara fruit is used to produce an organic acid for the tire, tanning, and herbal medicine industries.

Other organizations are also proposing fog-harvesting projects, with plans for as many as 20 nets in some Lima communities. There is some debate over which nets are the best design. Robert Schemenauer, who heads the Canadian nonprofit FogQuest based in Vancouver, pioneered fog harvesting with a

small experiment in the Chilean desert some 20 years ago. He developed the Standard Fog Collector, a simple, double-layer net system (double so as to improve drainage), used by Luna’s community, which removes 60% of the water in the air that hits it. Schemenauer’s model is now being used in projects in Oman, Namibia, Guatemala, and elsewhere.

Lummerich and Tiedemann now say they have improved on Schemenauer’s standard model. Alimón’s latest Eiffel Tower design, located above a community near Luna’s, is a three-dimensional system. The double layer is separated by strips of netting oriented 90 degrees to the main nets, capturing fog from the different wind directions. With this model, they say, each net achieves a yield of 300 liters per day, averaged over a year, and a best yield that tops 2650 liters per day, six times the yield of the Standard Fog Collector model—something Schemenauer disputes as “impossible.”

This July, at an international congress on fog research and fog collection, held in Münster, Germany, several new designs were pitched. They included a hexagonal “bee hive” system that can be lived in, an enhanced natural net fiber, and a Buckminster Fuller-inspired design.

No matter what the net design, this technology may not be the long-term solution that thirsty Lima residents yearn for, however. “Fog harvesting will only provide for people’s drinking, washing, and small-scale agricultural needs,” says Schemenauer. Once a community gets large enough to support businesses, such as cafes, swimming pools, and so on, the per capita daily water use goes up from about 45 liters per person to hundreds. Then, governments need to invest in piped water supply.

And fog nets won’t work just anywhere. On the Peru-Chile coast, nets must face the prevailing wind 500 to 700 meters above sea level and 5 to 10 kilometers inland to be most effective.

Finally, climate change brings more uncertainty. Typically, the ocean-warming phenomenon of El Niño, which occurs off the Pacific coast at roughly 5- to 7-year intervals, leads to more fog. But as these episodes become more intense in the future, scientists are divided over whether they will bring more fog or less. It could be less because the fog-carrying air may be so warm that the fog droplets will be too small to be captured by the nets, and the moist air will rise too high for the nets.

For the moment, however, Luna is betting that watering trees might just solve his water problem.

—GAIA VINCE

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Desperate measures.

At 4500 meters above sea level, Peruvian villagers are painting a mountain-side white to try to keep it cool. They plan on allowing water to trickle over the lightened landscape so it freezes and starts rebuilding a glacier. They are already seeing ice formation, but the plan has been met with skepticism.

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ARCHAEOLOGY

Using Old Insects to Sleuth Out New Clues to Ancient Cultures

From Moche burials in Peru to trash dumps in Akhenaten's Egypt, traces of insects are revealing ancient human societies and behavior

Criminal investigators have long recognized that insects can help solve crimes. As early as 1247 C.E., Chinese writer Sung T'zu described in a text how a magistrate solved the case of a peasant slain with a sickle. The magistrate lined up the suspects on a sunny day and asked each man to lay his sickle on the ground. As the magistrate waited, blowflies began swarming on one man's blade. Strongly attracted to carrion, the flies had detected traces of blood adhering to the murder weapon.

Today, entomologists routinely analyze insects at crime scenes to assist police. Now a wave of new studies extends this detective power back to the ancient past. Archaeologists studying diverse times and places are finding that insects can reveal details of ancient life available from no other source. By applying their knowledge of insect biology and behavior, entomologists have helped archaeologists to reconstruct the mortuary practices of the Moche in Peru, illuminate the sanitary—or unsanitary—conditions of an ancient Egyptian city, detail the environment of early humans in Britain 780,000 years ago, and probe the possibility of very early agriculture in Japan 9000 years ago. These new studies by no means exhaust the possibilities, says entomologist and forensic anthropologist Robert Pickering of Gilcrease Museum in Tulsa, Oklahoma. “Wherever people have been, there was an insect community either on them or with them,” he says. “By studying these insects we can create a clearer picture of the past.”

In a grisly example published in this month's issue of the *Journal of Archaeo-*

logical Science, archaeoentomologist Jean-Bernard Huchet of the University of Bordeaux 1 in France and entomologist Bernard Greenberg of the University of Illinois, Chicago, identified 1700-year-old fly remains to understand how the Moche people treated their dead. The Moche were largely maize agriculturalists who flourished between 100 C.E. and 700 C.E. and are known for depictions of human sacrifice in their art. Skeletal remains suggested that the Moche developed complex mortuary rituals, including delayed burials and reopened graves.

To probe such rituals, researchers analyzed insect remains. While excavating 57 graves at the site of Huaca de la Luna on Peru's north coast, CNRS archaeologist Claude Chauchat of the University of Paris in Nanterre found hundreds of small, barrel-shaped casings, each of which once



Plenty of pupae. Insect casings left in a grave more than 1700 years ago reveal the complex mortuary rites of Peru's Moche culture.

protected an insect pupa. Chauchat asked Huchet to identify nearly 200 puparia and other insect remains found in the grave of a young adult male who died between 1700 and 1900 years ago. “This was the grave richest in insects,” says Huchet, “with a lot of diversity of species.”

Huchet and Greenberg examined each puparium with a scanning electron microscope and compared diagnostic features—such as the contours of the respiratory slit—with those of known Peruvian species. The casings belonged to three taxa: houseflies (*Muscidae*); blowflies (*Calliphoridae*); and flesh flies (*Sarcophagidae*). Huchet also identified a fragment belonging to the *Trogiidae*, or carcass beetles, and determined that parasitic wasps hatched in some puparia.

He then gathered published data on the life history of these species. To find out when previously unstudied species began breeding on carrion, he conducted experiments in Peru by leaving out chunks of pork. Several of the flies had different timetables. One blowfly, for example, preferred laying eggs on the newly dead, whereas a certain species of housefly favored breeding on rotting, 2-week-old carcasses. The team drew up a timeline for insect succession on the ancient cadaver and concluded that the body had lain in the open for 1 month.

Why did the Moche hold off burying the dead? Huchet thinks they wanted to attract certain flies and their flesh-eating larvae as a way of releasing the human spirit after death, a practice followed by later Andean peoples. During the 17th century, indigenous Peruvians told a Spanish chronicler that “the cadaver must be left long enough to breed worms [fly larvae] so its anima can emerge and escape in the form of flies.” The idea fits well with Moche iconography. One surviving Moche ceramic bottle, for example, depicts hovering flies as Moche warriors led male prisoners off for sacrifice (see image above).

The Moche burials are “like a detective case,” says physical anthropologist John Verano of Tulane University in New Orleans,

CREDITS (TOP TO BOTTOM): ILLUSTRATION COURTESY OF DONALD MCCLELLAND; COURTESY OF JEAN-BERNARD HUCHET

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Lords of the flies. A Moche image shows insects hovering over prisoners being led to sacrifice.

Louisiana, “and Huchet is opening the door to a more nuanced reading of the dead.” The findings are “both dramatic and convincing,” agrees University of Bordeaux anthropologist Henri Duday, and point to “all the progress we can now expect” from studies of insects.

Taking out the trash in Amarna

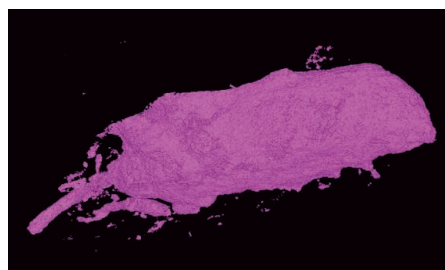
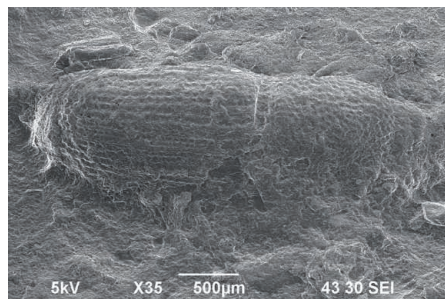
As studies of insects mature, they may complement—or contradict—other kinds of data. For example, tomb paintings depict the ancient Egyptian city of Amarna, founded in 1353 B.C.E. by the pharaoh Akhenaten, as a healthy, well-organized place. Archaeologists have even found a kind of landfill there, suggesting some form of organized trash disposal. But insect remains examined by paleoentomologist Eva Panagiotakopulu of the University of Edinburgh in the United Kingdom and her colleagues reveal a far more casual approach to garbage.

Archaeologists working in an affluent part of Amarna recovered insect remains and plant debris from deposits sandwiched between two house floors. The lower floor belonged to a small early house, but the upper floor was part of a large dwelling owned by Akhenaten’s chief charioteer, Ranefer, mentioned in door-post inscriptions. Because the area between the floors was sealed, the insects must have been deposited in the 25 years or so that Amarna was occupied.

In work published in the *Journal of Archaeological Science* in October 2009, Panagiotakopulu and Paul Buckland, a private paleoentomologist, identified 37 insect taxa from fragments of exoskeletons, puparia, and desiccated larvae. The beetles were largely pests of stored cereal grains, suggesting that someone had dumped badly infested grain on the first house floor. The researchers also found traces of swarming, breeding flies, mostly the housefly *Musca domestica*, which frequents “foul and dirty areas, especially when found in large numbers,” says Panagiotakopulu. The team also identified two beetle species that feed on teeming maggots.

To archaeologist Paul Nicholson of Cardiff University in the United Kingdom, the data strongly suggest that local residents threw their garbage into the early house after it was abandoned and before Ranefer built there. “It looks as if some of the refuse was just taken out and dumped,” says Nicholson, “so there must have been some really quite unpleasant areas in ancient Egyptian towns.”

In other cases, insects may offer details



Early pests. Microscopy (top) and CT scanning (above) show traces of maize weevils (center) in Japanese pots at least 9000 years old.

that fit with other evidence. For example, a well-preserved insect assemblage recently helped to reconstruct the ancient environment at Happisburgh Site 3 in England, occupied by early humans sometime between 780,000 and 1 million years ago. Previous evidence had suggested that hominins expanded into north-west Europe only when a warm, Mediterranean climate prevailed there. But paleoentomologist G. Russell Coope of the University of Birmingham, working with team leader Simon Parfitt of University College London, found that although most of the nearly 150 beetle species from Happisburgh samples were temperate species, a few inhabited boreal forests.

Coope then examined the modern climatic ranges of 34 of the species and calculated summer and winter temperatures in the overlap zone. He concluded that the mean temperature in Happisburgh’s coldest month fell to -3°C —colder than today. Such data give a far more local picture of climate than those obtained from oxygen isotope records found in ocean sediments, Coope says.

Together with other environmental data, Coope’s work indicates that Happisburgh’s early hominins ranged across a region whose

climate was similar to that of southern Scandinavia today. So “early Paleolithic humans were either a damn sight more sophisticated than we thought, being able to tolerate such cold conditions,” Coope says, “or alternatively, they could have been migratory, moving north only during the summer.”

Insect traces are also hinting at precocious human capabilities in a completely different time and place: an early Holocene site in Japan. Researchers studying 4000-year-old potsherds made by the ancient Jomon people have found tiny holes in the clay made by maize weevils, which commonly infest stored rice and barley today. More recently, a team led by archaeologist Hiroki Obata of Kumamoto University in Japan found seven such weevil-shaped cavities, each only about 3 millimeters long, in charcoal-bearing potsherds dated all the way back to 9000 years ago at Sanbonmatsu in southern Japan; unpublished data suggests the sherds may be even older.

In work announced in *Nature Precedings* in May 2010, Obata and his team injected each weevil hole with silicone to make a cast of the insect, which apparently got into the wet clay as the pot was being made. Scanning electron microscopy photographs and computed tomography scans suggest that the insect casts were those of maize weevils. “We were very surprised to find the oldest maize weevils,” says team member Aya Manabe of Kumamoto University.

But what were these weevils eating so long ago? The earliest widely accepted evidence for rice cultivation in Japan is rice phytoliths that date only to 4000 to 3000 years ago. So Obata and his team suggest that Sanbonmatsu’s weevils infested stores of wild foods, such as bamboo seeds or acorns.

Other researchers aren’t so sure. Sanbonmatsu lies in “an area of Jomon precocity,” says archaeologist Richard Pearson of the University of British Columbia, Vancouver, in Canada. As early as 11,000 years ago, Jomon families there resided in large villages and relied heavily on plant foods, as shown by an abundance of grinding stones. It’s possible that this precocity was fueled by some kind of early horticulture, says Pearson. “More evidence is clearly necessary,” he adds.

Whatever the Jomon people and their weevils were eating, entomologists agree that the surge of interest in very old insects bodes well for studies of the past. “I’m just staggered,” Coope concludes, “by what can be done with insects in archaeology.”

—HEATHER PRINGLE

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LETTERS

edited by Jennifer Sills

The Hazy Details of Early Earth's Atmosphere

IN THEIR REPORT "FRACTAL ORGANIC HAZES PROVIDED AN ULTRAVIOLET SHIELD FOR EARLY Earth" (4 June, p. 1266), E. T. Wolf and O. B. Toon base their fractal haze theory on the assumption that the Archean atmosphere was primarily N_2 . The Report includes no reference for this "prevailing view," and much evidence can be amassed against it. Geologists since Darwin have uniformly argued for a CO_2 -dominant atmosphere for the early Earth (1, 2).

The evolutionary roots of biochemistry draw on CO_2 and H_2 as the main nutrients of life. Life also requires the extra proton power afforded by chemiosmosis, an energy source that must have been available to emergent life (3–5). Primordial metabolism may have been based on minerals catalyzing the reaction between CO_2 and H_2 via the acetyl coenzyme-A pathway (6). Furthermore, data that enzymes involved in synthesizing sugars predate those that catabolize them (7) does not support the theory that catabolism of preformed organic molecules was the driver to life's emergence.

C. F. Chyba ("Countering the early faint Sun," Perspectives, 4 June, p. 1238) offers one alternative (i.e., autogenic) model: Wächtershäuser's surface metabolism (8). However, this hypothesis fails because the initial conditions invoked offer neither a natural protonmotive force to drive biosynthesis, nor a compartment for its focus. The alkaline hydrothermal hypothesis does address this (8) and other aspects of life's onset, in a model that leads logically to the acetyl coenzyme-A pathway, without resorting to contingency (9).

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References

1. B. J. Wood *et al.*, *Science* **248**, 337 (1990).
2. H. Ohmoto *et al.*, *Nature* **429**, 395 (2004).
3. M. J. Russell *et al.*, *Terra Nova* **5**, 343 (1993).
4. W. Martin, M. J. Russell, *Philos. Trans. R. Soc. London Ser. B* **362**, 1887 (2007).
5. W. Nitschke, M. J. Russell, *J. Mol. Evol.* **69**, 481 (2009).
6. I. A. Berg *et al.*, *Nat. Rev. Microbiol.* **8**, 447 (2010).
7. R. F. Say, G. Fuchs, *Nature* **464**, 1077 (2010).
8. G. Wächtershäuser, *Microbiol. Rev.* **52**, 452 (1988).
9. M. J. Russell, W. Martin, *Trends Biochem. Sci.* **29**, 358 (2004).



Titan's haze. Early Earth's atmosphere may have resembled that of Saturn's moon Titan.

atmosphere dominated by N_2 and requiring greenhouse gases in addition to CO_2 to keep the young Earth warm.

Admittedly, achieving high methane concentrations before organic material existed on Earth is difficult (4). However, methane concentrations of 1000 parts per million or higher supplied by methanogens are predicted for the postbiotic Earth (5). When the CH_4/CO_2 ratio rose above 0.1, N_2 – CH_4 photochemistry could proceed (6), creating the ultraviolet-shielding fractal organic haze we described. Ammonia could have been protected from photolysis beneath the haze, yielding an atmosphere rich in both CH_4 and NH_3 , thus making it possible for these inorganic material to yield organic compounds (Miller-Urey chemistry), as we noted in our Report.

Although our work makes no attempt to address the specific biochemical mechanisms that lie at roots of life, we do address important questions regarding the atmospheric composition and climate of the Earth at the time when life first flourished. Recent studies (6, 7) along with the evidence amassed against a CO_2 -rich atmosphere indicate that the Archean was at least mildly reducing. Whether the

very first life was formed as a direct result of chemical reactions of inorganic material, as indicated by Miller-Urey chemistry, is up for debate, but given the emerging new picture of the Archean, surely Miller-Urey chemistry would have proceeded at some point early in the Earth's history. The haze chemistry itself would have produced organics at a rate that would likely dwarf the production of organics from the hydrothermal vent systems favored by Russell (5). Laboratory studies have confirmed that complex organics are readily produced in early Earth-like environments

Response

RUSSELL ARGUES THAT A TITAN-LIKE VIEW of the early Earth is inaccurate and that a CO_2 -dominated atmosphere is more likely. This view dominated thinking in the 1980s and 1990s, but has since been in decline. Geochemical arguments have been made supporting low CO_2 abundances. Rosing *et al.* argue that the presence of magnetite in banded-iron formations constrains atmo-

spheric CO_2 to a mere 3 times the present atmospheric level (1). Moreover, vigorous plate tectonics would likely have sequestered most CO_2 within the mantle (2). Russell assumes that N_2 was not likely the dominant gas. However, Goldblatt *et al.* (3) suggest that a higher fraction of Earth's total nitrogen budget was present in the young atmosphere than today. In contrast to Russell's assumptions, these recent studies point toward a young

containing N_2 , CO_2 , CH_4 , and H_2 (8). The organic haze particles may have been edible and would have precipitated into the young oceans, creating an organic soup consistent with Miller-Urey (9).

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References

1. M. T. Rosing, D. K. Bird, N. H. Sleep, C. J. Bjerrum, *Nature* **464**, 744 (2010).
2. N. H. Sleep, K. Zahnle, *J. Geophys. Res.* **106**, 1373 (2001).
3. C. Goldblatt *et al.*, *Nat. Geosci.* **2**, 891 (2009).
4. J. F. Kasting, *Precambrian Res.* **137** 119 (2005).
5. A. A. Pavlov, L. L. Brown, J. F. Kasting, *J. Geophys. Res.* **106** 23267 (2001).
6. J. D. Haqq-Misra, S. D. Domagal-Goldman, P. J. Kasting, J. F. Kasting, *Astrobiology* **8** 1127 (2008).
7. F. Tian, O. B. Toon, A. A. Pavlov, H. De Sterck, *Science* **308** 1014 (2005).
8. H. L. Dewitt *et al.*, *Astrobiology* **9** 447 (2009).
9. M. G. Trainer *et al.*, *Astrobiology* **4** 409 (2004).

Response

RUSSELL PROPOSES THAT EARLY EARTH'S atmosphere contained primarily CO_2 and lacked N_2 . For this idea to make sense, one must explain how volcanic outgassing or comet/asteroid impact delivery of the early atmosphere's constituents could provide CO_2 yet sequester N_2 .

In my Perspective, I focused on the implications of the Wolf and Toon Report for early Earth's greenhouse; I only briefly touched on its implications for the origin of life. Wolf and Toon's model removes one of the long-standing objections to an early atmosphere with substantial methane and ammonia, and therefore to the Miller-Urey organic "building block" approach to the origin of life. Their model cannot speak to other important objections to the building-block hypothesis. I contrasted the Miller-Urey picture with metabolism-first theories in which life originates with autocatalytic cycles (cycles in which the product of the chemical reaction is also a reactant) that use inorganic carbon such as CO_2 (1). Clearly, metabolism-first theories are now a burgeoning subfield of their own (2). More recent discoveries of hydrothermal venting far from ocean ridges (3) have propelled alkaline-environment metabolism-first theories, such as the one Russell describes, into the spotlight (4).

Huber and Wächtershäuser (5) showed that the crucial reaction in the acetyl coenzyme-A pathway that Russell favors could have occurred prebiotically (before life existed), supporting the idea that metabolism

could have evolved from prebiotic chemistry. A centrality of the acetyl coenzyme-A path to the origin of life is therefore broadly consistent with the Wächtershäuser approach, although there are important differences, which Russell argues favors his model (6). The alkaline model for life's origin may also be relevant to Jupiter's moon Europa (7).

Complex environmental questions about early Earth and the origin of life may well have composite answers. Researchers need to understand the strengths, weaknesses, and possible complementary roles of multiple approaches to the problem.

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References

1. G. Wächtershäuser, *Microbiol. Rev.* **52**, 452 (1988).
2. G. D. Cody, J. H. Scott, in *Planets and Life*, W. T. Sullivan, J. A. Baross, Eds. (Cambridge Univ. Press, Cambridge, 2007), pp. 174–186.
3. D. S. Kelley *et al.*, *Nature* **412**, 145 (2001).
4. W. Martin, J. Baross, D. Kelley, M. J. Russell, *Nature Rev. Microbiol.* **6**, 806 (2008).
5. C. Huber, G. Wächtershäuser, *Science* **276**, 245 (1997).
6. M. J. Russell, *Science* **302**, 580 (2003).
7. K. P. Hand, C. F. Chyba, J. C. Priscu, R. W. Carlson, K. H. Nealson, in *Europa*, R. T. Pappalardo, W. B. McKinnon, K. Khurana, Eds. (Univ. of Arizona Press, Tucson, AZ, 2009), pp. 589–629.

Funding for Chinese Collaboration

IN THEIR EDITORIAL “CHINA’S RESEARCH CULTURE” (3 September, p. 1128), Y. Shi and Y. Rao describe an example of the rampant problems in China’s research funding allocation, namely the selection of recipients for “mega-project grants.” I often hear stories about very expensive equipment left packed in hallways or labs for years without being used. The funding agencies often have very strict guidelines for using the funding on salaries, even though a research group’s ability to hire the talent they need is often the most important factor in the success of the research program.

China’s funding strategy for overseas Chinese scientists is also problematic. As part of an Asia-wide trend, China has been trying to recruit talents from overseas (1). To attract established overseas Chinese researchers with advanced education from western countries, China has devoted billions of Chinese yuan to talent programs [such as the Thousand Talent program (2) recently established by the central government] that require overseas scholars to relocate to China to accept prestigious full-time positions. However, many recipients of these awards cannot relocate because of practical and family obligations.

China should focus instead on grants that

fund collaborative research between overseas Chinese scholars and their peers in China. Collaborative programs are more cost-effective and more practical for those who cannot relocate. One such program is the Joint Research Fund (JRF) for Overseas Chinese Scholars and Scholars in Hong Kong and Macao, administered by the National Natural Science Foundation of China (NSFC). In 2006, a mere 0.7% of the NSFC budget was allocated to this worthy program (3). In 2008, the maximum grant was reduced from 400,000 Chinese yuan over 3 years to 200,000 Chinese yuan over 2 years (4, 5). By dedicating such a small budget to this program and others like it, China misses an opportunity to engage overseas Chinese scholars and benefit from their contributions to the country’s research and education.

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References

1. A. S. Huang, C. Y. H. Tan, *Science* **329**, 1471 (2010).
2. Thousand Talent program [www.1000plan.org (in Chinese)].
3. National Natural Science Foundation of China, Financial Statistics of NSFC in 2006 (www.nsf.gov.cn/english/11st/index.html).
4. National Natural Science Foundation of China, Fund for Talented Professionals (NSFC, 2008), p. 17; www.nsf.gov.cn/english/06gpp/pdf/2008/051.doc.
5. National Natural Science Foundation of China, Funds for Talented Professionals (NSFC, 2007), p. 145; www.nsf.gov.cn/english/06gpp/pdf/2007/031.pdf.

CORRECTIONS AND CLARIFICATIONS

Brevia: “Pulsar discovery by global volunteer computing” by B. Knispel *et al.* (10 September, p. 1305). The Einstein@Home data are transferred to the Albert Einstein Institute (not Leibniz Universität) in Hannover, Germany.

Reports: “Prediction of individual brain maturity using fMRI” by N. U. F. Dosenbach *et al.* (10 September, p. 1358). In Fig. 2, the labels in the bottom-right image (anterior view) were incorrect. The “L” and “R” labels should be switched.

Reports: “Unprecedented restoration of a native oyster metapopulation” by D. M. Schulte *et al.* (28 August 2009, p. 1124). Reference 19 was incorrect. The correct reference is “K. Greenhawk, T. O’Connell, L. Barker, Oyster Population Estimates for the Maryland Portion of Chesapeake Bay 1994–2006 (Maryland Department of Natural Resources, 2007), Table 7; www.dnr.state.md.us/fisheries/oysters/mtgs/MDOysterPopEst_07_27_07.pdf.”

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

NEUROSCIENCE

Seeing Through Incapacities

Christof Koch

The heaventrees of stars hung with humid
nightblue fruit (1)

This line from James Joyce's *Ulysses* comes unbidden to mind upon reading *The Mind's Eye*, the latest collection of brain-gone-wrong case studies by the British-born but consummate New Yorker neurologist Oliver Sacks. Both authors enjoy mashing up evocative sentences dealing with distinct domains, here the mental and the neuronal. But unlike *Ulysses*, *The Mind's Eye* can be read in a single sitting.

One of the fathers of modern thought, René Descartes, was among the first to link a specific structure in the brain (the pineal gland) to a specific attribute of the mind (the soul). In modern language, Descartes proposed that this gland, part of the endocrine system, is the neuronal correlate of consciousness. We, his intellectual heirs, know better and smugly think "how silly." Yet the principle of localization is alive and well. This is one of the chief take-home lessons of *The Mind's Eye*.

Destruction of tissue in the back of the cerebral cortex by a hemorrhage, tumor, or atrophy leaves one unable to recognize objects by sight. There is nothing wrong with the eyes, but a hat stand becomes an unknown and alien thing, a construct of lines that doesn't give rise to any meaningful object. For the highly literary patients of Sacks, this loss extends to letters and words, which lose their sense. Seemingly paradoxically, this inability to read or alexia does not imply agraphia, the inability to write. (Any medical syndrome preceded by "a" spells trouble, as it signifies the absence of a skill.) Lilian, a patient introduced on the book's first page, carries on an extended correspondence but is unable to read any of her letters. Early on in her slowly spreading posterior cortical atrophy, she can still laboriously read short words, letter by letter, as a child does, but this eventually fails.

Howard is an accomplished Canadian novelist. He discovers one morning that his trusted *Globe and Mail* is now printed in

an undecipherable script—perhaps Serbo-Croatian, Korean, or hieroglyphs. A stroke in a limited region of his visual cortex destroys his ability to recognize text, faces, colors, and objects. Yet, as does Lilian, he retains his writing skills, living proof that reading and writing are complementary yet distinct skills. The powers of the brain to recuperate, to adapt and exploit other (perhaps previously neglected) senses, and to invent coping strategies can be considerable, even in the elderly. Howard becomes an avid consumer of audiobooks and text-to-speech software. Challenged by his condition, he writes a successful alter-ego crime novel, with the protagonist detective waking up in hospital with both alexia and amnesia.

Other chapters deal with how people adapt to blindness, with aphasia (the inability to understand or generate speech), and with face blindness (the loss of the facile and automatic identification of faces). For those

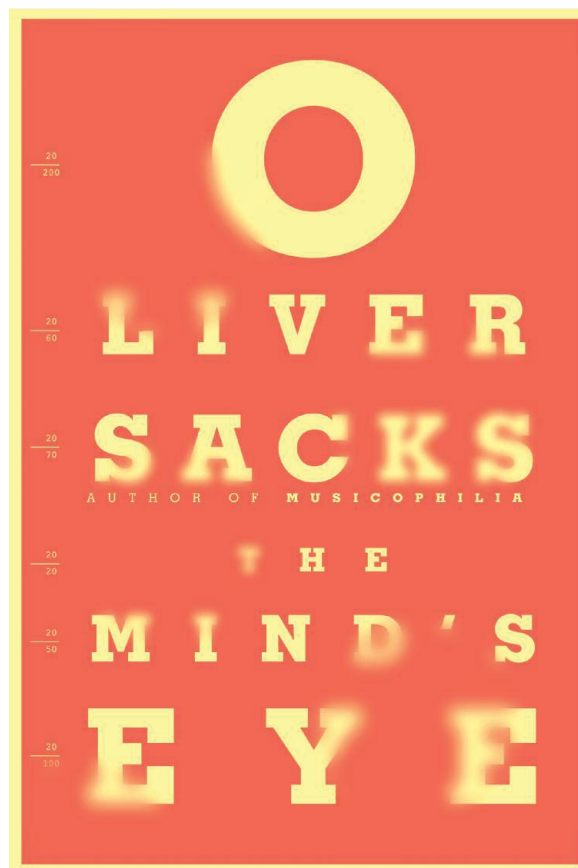
afflicted by the latter, the faces of loved or familiar ones all look equally indistinguishable to the afflicted, like the guy ahead in the supermarket checkout queue. This incapacity has far-reaching consequences, for the affected individual will often withdraw from society to avoid embarrassing situations.

For students of the mind-body conundrum, these brilliantly described cases provide the definite answer to Nietzsche's rhetorical question in *The Gay Science*, "And as for sickness: are we not almost tempted to ask whether we could get along without it?" (2). Sacks does an excellent job of contextualizing his patients' symptoms and their underlying causes within the contemporary neuroscientific and neurological literature. What elevates this clinical material into the realm of high literature are the author's superb observational skills, without equal. Sacks is an avid witness of the human condition, demonstrating how, in examining the means by which people deal with disease, one can gain wisdom about life. This becomes most apparent in the central and longest chapter. That tells Sacks's own story, drawn from his diary (along with pencil sketches) detailing his experience with ocular melanoma.

First, the dreaded news uttered by the doctor, cancer. Then, the excruciating and nightmarish wait until the Christmas and New Year's holidays are over for the surgical removal of the tumor with focused radiation that will, he and his doctors hope, save most of his retina. The surgery leaves him with a large black hole in his right eye. Sacks's amazing powers to describe, to evoke, to call forth, to fascinate, and to associate outdo themselves. He celebrates his changed visual experience in a riot of coruscated imagery and an exuberance of words. He vividly describes how this black nothingness, his scotoma (the region of visual space destroyed by the tumor), fills in when looking at a blue sky or at a patterned

The Mind's Eye
by Oliver Sacks

Knopf, New York,
2010. 281 pp. \$26.95.
ISBN 9780307272089.



Effective communication after all.

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brick wall. The same processes that fill in the blind spot (the “hole” in the eye we all have) are at work here, compensating for the loss with data from the surrounding regions. Like a kid discovering what can and can’t be done with a new toy, Sacks describes the strengths and the limits of this filling-in. He can amputate his leg by moving it into his scotoma; yet when he wiggles and moves it, the sensory-motor feedback from what he can’t see renders the leg visible in a ghostly sort of way. Conversely, a flock of birds that enters his scotoma abruptly disappears, only to emerge intact on the other side. His reaction to further operations and an abrupt bleeding into the weakened eye is to meditate on the loss of perceived depth associated with stereo vision.

Sacks is not a religious man. Yet when reading *The Mind’s Eye*—most, but not all, of whose pages deal with disease, pain, or loss—the reader comes away with numinous feelings of wonder, mysticism, and gratitude. What more can one want from any book?

References

1. J. Joyce, *Ulysses* (Shakespeare and Company, Paris, 1922).
2. F. Nietzsche, *Die fröhliche Wissenschaft* (Fritzsch, Leipzig, 1887).

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MOLECULAR BIOLOGY

An RNA Whirl

Irene A. Chen

For anyone interested in understanding how life could begin, the new millennium has yielded a treasure trove of exciting discoveries. Research into the possible origins of life can be classified into three major themes, all of which have seen remarkable progress in recent years.

First, the right habitat and chemistry must exist. The past decade has seen the discovery of hundreds of exoplanets, and now several lines of evidence point to the presence of water on Mars, including liquid water in the past (1). In addition, chemical reactions that mimic prebiotic conditions exhibit remarkable selectivity under certain conditions, suggesting ways around the old bugaboo of non-specific synthesis (2).

Second, order and reproduction must emerge through physical or chemical mechanisms. Recent work demonstrates how

simple membranes can spontaneously self-assemble, grow, divide, and even compete with one another (3–5). Much progress has also been made on the old puzzle of the emergence of homochirality, which turns out to be remarkably easy: for example, simply grinding crystals of an amino acid can essentially convert a mixture to one-handedness (6). Still, many unexplored frontiers remain regarding the initial emergence of apparently biological properties.

Third, after the first replicating, information-carrying entities arose, Darwinian evolution took over as the dominant process that eventually led to the diversity of living organisms we see today. Promising work has been directed toward building primitive cells that could be capable of replicating and evolving. Although precise historical details of the particular origin of life on Earth are probably unknowable, most scientists agree that a world existed in which RNA performed the duties of both genes and enzymes. This RNA world in turn evolved into the DNA-RNA-protein world of today (7). Michael Yarus’s *Life from an RNA World* offers an engaging introduction to the subject. Remarkably, as Yarus (a molecular biologist at the University of Colorado) points out, in the 1960s Leslie Orgel, Francis Crick, and Carl Woese each postulated an RNA world. They based their suggestions on the discovery that RNA could fold with structural complexity reminiscent of proteins. The theory provided an elegant solution to the chicken-and-egg mutual dependence of DNA and proteins for replication, but no one knew for certain that RNA could catalyze reactions. That was confirmed in the 1980s, with the discovery of the first ribozymes.

Many consider the structure of the ribosome—elucidated in 2000 and the subject of the 2009 Nobel Prize in chemistry—to be the smoking gun of the RNA world, because it demonstrates that catalysis of translation is performed by the ribosome’s RNA component whereas the proteins serve primarily as a structural scaffold. Until recently, most researchers believed that several chemical features made RNA a suboptimal genetic material. Thus, people speculated that a simpler, more-robust nucleic acid preceded RNA. However, a recent elegant synthesis of ribonucleosides from prebiotic reactants has resurrected the idea that RNA might even have been the first genetic material (8).

The recent discoveries make Yarus’s book particularly timely, especially as a

light-hearted introduction for scientifically minded readers outside the field. His chatty prose conveys the voice of a tour guide on a journey through the RNA world, introducing essential evolutionary and molecular biology

and pointing out must-not-miss attractions. Even members of the origins-of-life community may appreciate his whimsical explanations of familiar phenomena, and later chapters contain subtle material more suitable for specialists (e.g., direct physical interactions of amino acids with their codons and

anticodons). Each chapter ends with a short but helpful reading list, which references authoritative reviews or books and research articles of special significance.

The author’s discussion of how natural selection can lead to complexity is particularly eloquent. Although that topic has been dealt with in countless books intended for a broad audience, the continued rise of creationist sentiment signals an ongoing need for advocacy on behalf of evolution by natural selection. Yarus revisits classic evidence and thought experiments that demonstrate how complex structures (such as the eye) could evolve and why such structures are not irreducibly complex. Indeed, biological complexity is often in the eye of the beholder—our inability to intuit the path that led to a particular biological property should be taken by default as a reflection of our own limited intuition, not as the absence of a path. Natural selection is a powerful mechanism for carving paths toward apparently complex properties. At the earliest stages of life, before replicators came onto the scene, physico-chemical mechanisms must have played this role. Building intuition and accumulating evidence for such paths remains one of the great intellectual and scientific challenges.

References

1. M. C. Malin, M. H. Carr, *Nature* **397**, 589 (1999).
2. A. Ricardo, M. A. Carrigan, A. N. Olcott, S. A. Benner, *Science* **303**, 196 (2004).
3. P. Walde, R. Wick, M. Fresta, A. Mangone, P. L. Luisi, *J. Am. Chem. Soc.* **116**, 11649 (1994).
4. M. M. Hanczyc, S. M. Fujikawa, J. W. Szostak, *Science* **302**, 618 (2003).
5. I. A. Chen, R. W. Roberts, J. W. Szostak, *Science* **305**, 1474 (2004).
6. D. G. Blackmond, *Cold Spring Harbor Perspect.* **2**, a002147 (2010).
7. R. Gestland, T. Cech, J. Atkins, Eds., *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 3, 2006).
8. M. W. Powner, B. Gerland, J. D. Sutherland, *Nature* **459**, 239 (2009).

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Life from an RNA World The Ancestor Within

by Michael Yarus

Harvard University Press,
Cambridge, MA, 2010.
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HEALTH CARE DELIVERY

Open mHealth Architecture: An Engine for Health Care Innovation

Deborah Estrin^{1*} and Ida Sim²

Chronic diseases like diabetes, asthma, and obesity account for 46% of global disease burden (1). The traditional model of episodic care in clinic and hospital-based settings is suboptimal for improving chronic disease outcomes (2). Mobile communication devices, in conjunction with Internet and social media, present opportunities to enhance disease prevention and management by extending health interventions beyond the reach of traditional care—an approach referred to as mHealth (3). However, mHealth is emerging as a patchwork of incompatible applications (“apps”) serving narrow, albeit valuable, needs, and thus could benefit from more coordinated development (4). A public-private partnership to define and instantiate an “open” mHealth architecture (described below), in the context of economic incentives and enabling policies, could support medical discovery and evidence-based practice about managing and preventing chronic disease.

Why mHealth?

Development and treatment of chronic diseases take place in daily life outside of traditional clinical settings. To determine and adjust treatment for these diseases, clinicians depend heavily on patient reports of symptoms, side effects, and functional status. Typically, patients report at clinic visits that are months apart, and recall accuracy can be highly variable (5). mHealth makes it feasible for patients to collect and share relevant data at any time, not just when they happen to visit a clinic, allowing more rapid convergence to optimal treatment. For example, a patient with epilepsy can self-report on drugs and dosages taken and the number and severity of seizures and side effects. The app sends this data in real time to the clinician, who can look for patterns of response and guide the patient to titrate his medications over weeks instead of months.

mHealth apps can contribute to a rapid,

learning health system, but this may be difficult if each app is built as a closed application with its own proprietary data format, management, and analysis. Such a “stovepipe” or “siloe” approach fundamentally limits the potential of mHealth by impeding data-sharing with other apps and with electronic and personal health records (EHRs and PHRs). Inefficiencies and lack of innovation plague health information technology (IT) systems that are closed and rigid (6). For example, a patient who is diabetic, hypertensive, and suffering from depression is unlikely to sustain use of multiple, siloe, noncommunicating, disease-specific apps that each monitor diet and medications. An open architecture built around shared data standards and the global communication network already in place to support interoperable voice and data transfer can promote the scaling, coherence, and power

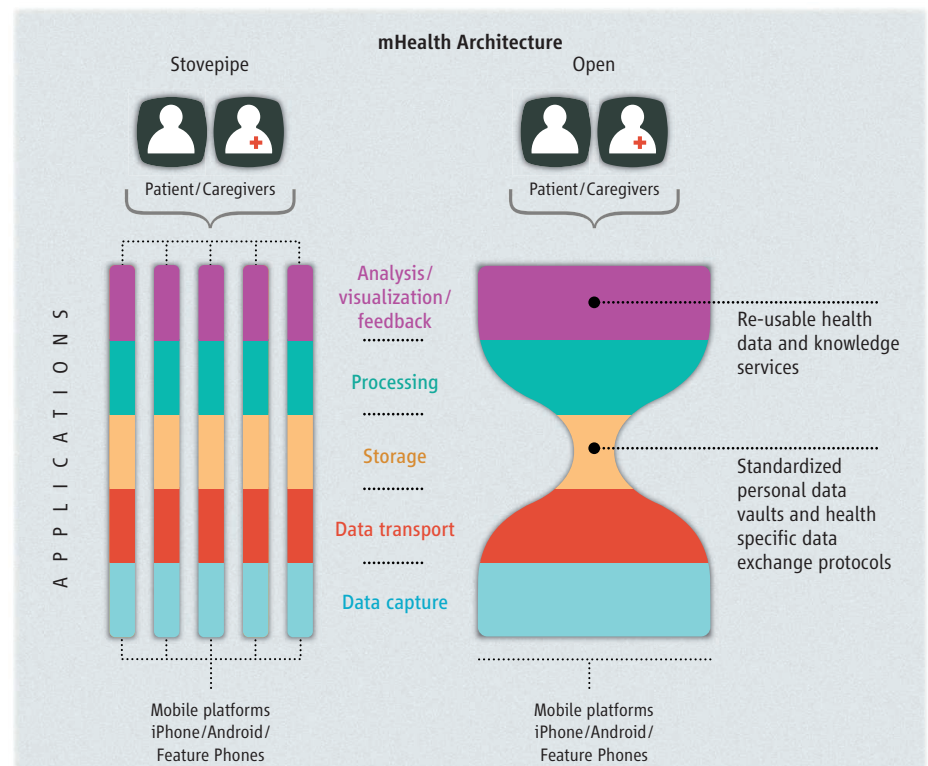
Standardized interfaces and shared components are critical for realizing the potential of mobile-device-enabled health care delivery and research.

of mHealth. Such an architecture should complement broader ongoing developments for scalable and sustainable health information systems, including various national (7, 8) and international (9, 10) initiatives.

Open Architecture Benefits

In an open architecture, components have well-defined, published interfaces that allow interconnection and use in ways other than as originally implemented or intended (11). They allow interested parties to expand the functionality of the system without modifying existing components.

Open architectures act as innovation infrastructure much like transportation, telecommunications, and financial systems. Although not perfect (for example, because of weakness in built-in security), the Internet’s open architecture sparked unprecedented cycles of innovation across all sectors of the economy.



mHealth architecture: Stovepipe versus Open. The narrow waist of the open hourglass will include at least health-specific syntactic and semantic data standards; patient identity standards; core data processing functions such as feature extraction and analytics; and data stores that allow for selective, patient-controlled sharing. Standards should be common with broader health IT standards whenever possible.

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The Internet's "hourglass" architecture, with its "narrow waist" a common Internet protocol for transferring data, and open interfaces on all sides (e.g., multiple transmission technologies and applications), was critical to its success (12). Just as this architecture allowed "killer" (i.e., transformative) apps such as the Web to emerge, a similar open, mHealth architecture (see the figure), with interoperability standards [e.g., (10)] enabling a narrow-waisted shape, may promote chronic disease prevention and management as a driving "killer app."

Clinical Care Research and Innovation

Open mHealth architecture may encourage innovation in health practices by easing application development. Shared standards and reusable components may enable rapid authoring, integration, and evaluation of personal data capture for clinical care and research. Hospitals, accountable care organizations, and public health practitioners could mix and match from a rich, flexible set of data acquisition and analysis components to configure custom apps [e.g., what symptoms to monitor, when, where, and how, or what data sources to incorporate (8)].

The experience base in mHealth is nascent, so research is needed to determine effective applications. Open architecture mHealth apps could be iteratively specialized to maximize usability across particular populations, diseases, and treatment protocols, and many underlying modules could be reused across applications. If architecture is coupled with a mechanism for updating shared components based on ongoing user evaluations, and appropriate incentives and policies exist, then best practices may be quickly propagated across apps to promote shared learning for mHealth usage across a broad range of health objectives.

By opening mHealth architecture, and thus lowering the barriers to entry, a broad community of patients, clinicians, family, and others could be involved in collaborative, participatory design of mHealth apps, providing new tools for extending care into the daily lives of families and communities. mHealth can amplify benefits of such real-world contexts in which "health happens," while exploring possibilities among youth and other early technology adopters (13). With the right architecture and shared building blocks, stakeholders could strive to create mHealth apps that protect patient privacy while using emerging data and identity standards to achieve semantically coherent interoperation with other systems.

Such accessibility to tools and popula-

tions with ubiquitous mobile devices could advance clinical care and research at a scale and resolution never before affordable. For example, despite the prevalence of depression, we lack good evidence on the long-term comparative effectiveness of antidepressants (14). In 2005, 27 million U.S. patients were prescribed antidepressants (15). Suppose every patient prescribed an antidepressant were invited by text message to participate in an antidepressant study. Patients would download an app that secures informed consent, prompts collection of standardized data (e.g., depressive symptoms, side effects, and daily activity levels), sends data to the EHR for clinician review to inform medication titration, retrieves predefined covariates from the patient's EHR (e.g., age and other medications), and anonymizes the data before sending it to the study coordinator. If only 1 out of 250 antidepressant patients in the U.S. consented to this study, the more than 100,000 enrolled patients would exceed the total number of patients enrolled in all antidepressant studies conducted worldwide as registered in ClinicalTrials.gov since 2005, when prospective trial registration first took hold (16).

Internationally, mHealth projects have focused on community health workers rather than on direct engagement of patients and have been limited by small scale and lack of technical and policy coordination (5, 17). A patient-centric, global, open mHealth initiative could usher in transnational health promotion and research projects that are prohibitively costly today.

Why Now?

Siloed approaches have plagued development of health information systems, creating expensive barriers to entry and hampering health care innovation (6). Lacking an open architecture, new entrants to mHealth, including app developers, care providers, and patient communities, would proliferate solutions based on this dominant, siloed framework. But given the early stages of development, there are relatively few mHealth legacy systems and entrenched silos to overcome. Thus, this is an opportune time for a new approach, in which those same parties could leverage an open architecture to expand mHealth capacity. In the developing world, where there are few legacy health IT systems in general, the benefits of this approach may even extend beyond mHealth to the broader care system.

A modest, coordinated investment is needed to nucleate a reference implementation of this architecture informed by early pilots. A shared underlying architecture will

enable much-needed scalable, affordable, and systematic research to determine which apps work best and for what populations and diseases. Defining the proper contents and interfaces to the narrow waist for mHealth is not trivial, but neither is it a complex, technical research challenge. To oversee the myriad technical, governance, and business issues, a public-private partnership is needed to balance public and commercial interests and combine the best of technology development and health care expertise. This could foster an economically and socially rewarding mHealth marketplace that uses the best health care evidence.

Approximately 25 years ago, government and industry invested in expanded access at a crucial time in the Internet's development (12). The resulting networks and ubiquity of access provided fertile ground for technologies, ideas, institutions, markets, and cultures to innovate. The payoff from this investment created a commercially viable and largely self-governing ecosystem for innovation. The same can be done for global health. Government, commercial, and nongovernmental entities involved in health IT and innovation should cooperate to define and instantiate architecture, governance, and business models and to steer initial mHealth investments into open architecture.

References and Notes

1. World Health Organization, Facts related to chronic disease; www.who.int/dietphysicalactivity/publications/facts/chronic/en.
2. E. H. Wagner *et al.*, *Health Aff.* **20**, 64 (2001).
3. mHealth Alliance; www.mhealthalliance.org.
4. Earth Institute, *Barriers and Gaps Affecting mHealth in Low and Middle Income Countries: A Policy White Paper* (mHealth Alliance, Washington, DC, 2010).
5. A. A. Stone *et al.*, *The Science of Real-Time Data Capture: Self Reports in Health Research* (Oxford Univ. Press, Oxford, 2007).
6. J. D. Kleinke, *Health Aff.* **24**, 1246 (2005).
7. National Health Information Network, www.hhs.gov/healthit/healthnetwork/background.
8. Community Health Data Initiative, www.hhs.gov/openplan/opengovernmentplan/initiatives/initiative.html.
9. Health Metrics Network and World Health Organization, *Framework and Standards for Country Health Information Systems* (WHO, Geneva, 2008).
10. HL7 International, www.hl7.org.
11. Open mHealth; <http://openmhealth.org>.
12. National Research Council, *The Internet's Coming of Age* (National Academy Press, Washington, DC, 2001).
13. A. Lenhart *et al.*, *Teens and Mobile Phones* (Pew Research Center, Washington, DC, 2010).
14. A. Cipriani *et al.*, *Lancet* **373**, 746 (2009).
15. M. Olsson, S. C. Marcus, *Arch. Gen. Psychiatry* **66**, 848 (2009).
16. D. A. Zarin, T. Tse, N. C. Ide, *N. Engl. J. Med.* **353**, 2779 (2005).
17. M. Mars, R. E. Scott, *Health Aff.* **29**, 237 (2009).
18. Supported by the U.S. National Science Foundation and the Mount Zion Health Fund. The authors thank M. Hansen, N. Ramanathan, M. Swiernik, F. Wells, J. Wing, and Y. Sun.

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CANCER

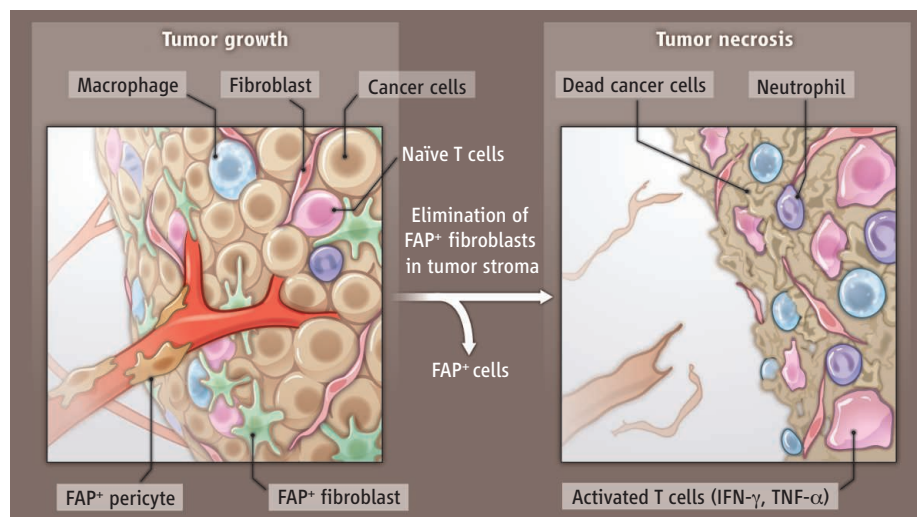
Awakening Immunity

Hans Schreiber and Donald A. Rowley

Cancer cells are embedded in stroma, the connective tissue framework of solid tumors. It consists of nonmalignant hematopoietic and mesenchymal cells, as well as extracellular matrix. Whether stromal cells have an essential role in cancer development and growth has been long debated. On page 827 of this issue, Kraman *et al.* (1) show that deleting a subpopulation of stromal fibroblasts arrests the growth of a solid tumor, an effect that depends on an immune response to the tumor. These results agree with other studies suggesting that immunizing against fibroblasts in tumors can unmask an immune response to cancer (2, 3).

Stromal cells and cancer cells depend on each other for mutual paracrine stimulation. Stromal fibroblasts are probably required for cancer cells to survive and grow (4), but why does their elimination trigger an immune response to the cancer cells? A clue may come from a particular subtype of fibroblast whose removal elicits this response. Fibroblasts from malignant solid tumors show increased expression of genes that are repressed in other tissue fibroblasts (5), particularly the genes encoding the cytoskeletal protein α -smooth muscle actin (α -SMA) and fibroblast activation protein (FAP), a serine protease. Both proteins are also expressed on pericytes (α -SMA⁺ and FAP⁺), stromal cells that reside at the interface between tumor endothelium and surrounding tissue. Stromal cells expressing these markers may suppress the immune response to tumors as a consequence of producing massive amounts of stromal cell–derived factor–1 (SDF-1/CXCL12). SDF-1 attracts regulatory T cells (CD4⁺ subtype) into the tumor (6). It also causes random movement of effector T cells, which interferes with T cell–tumor cell interaction and ultimately hinders tumor destruction (7).

Eliminating neutrophils also produces antitumor immune effects similar to those caused by eliminating FAP⁺ stromal cells (8, 9). Neutrophils release matrix metalloproteinase 9 and elastase, which enzymatically “free” stromal fibroblast progenitor cells from bone marrow and perivascular reservoirs (10), allowing them to follow the SDF-1 cytokine gradient into the tumor.



Tumor destruction. Removing FAP⁺ fibroblasts and pericytes damages the blood supply and causes some cancer cells to die. The resulting damage-associated signals, together with antigens released by dying cancer cells, triggers the production of cytokines (IFN- γ and TNF- α) by cancer antigen-specific T cells in the tumor. This results in the destruction of the remaining cancer and stromal cells by the immune system.

Metalloprotease released from neutrophils also catalyzes the release and activation of latent transforming growth factor- β (TGF- β) from the extracellular matrix. TGF- β 1 activates stromal fibroblasts (α -SMA⁺ and FAP⁺), causing them to produce immunosuppressive SDF-1. TGF- β 1 also prevents the initiation of effector T cell responses. Furthermore, depending on the stimulus, neutrophils and other leukocytes can themselves produce large amounts of TGF- β 1 (11).

Kraman *et al.* deleted FAP⁺ stromal cells from mice bearing solid tumors that arose from injected lung cancer cells. These cancer cells were engineered to express ovalbumin, a xenoantigen capable of eliciting an immune response. The removal of FAP⁺ fibroblasts did not alter the number or subtypes of tumor-infiltrating T cells, but did result in their activation and secretion of the pro-inflammatory cytokines interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α) (see the figure). But in an actual tumor, what type of cancer cell antigens would be suitable for effective tumor destruction by the immune system? Kraman *et al.* emphasize the importance of unmutated self-antigens on cancer cells as effective elicitors. Although targeting by the immune system of viral antigens such as EBNA3 can eradicate large, bulky masses of lymphomas induced by Epstein Barr virus, it is not known what the critical characteristics

of an antigen are that allow effector T cells to destroy large, established solid tumors or aggregates of cancer cells that have dispersed from the primary tumor to the rest of the body. EBNA3 is not expressed on normal cells and is essential for the cancer cells to remain malignant. The closest correlate to antigens on cancers that are not associated with viruses are tumor-specific mutant proteins that are essential for maintaining malignancy.

In this context, FAP as a self-antigen may be problematic because it is expressed on nonmalignant cells and its expression can be lost (12). Nevertheless, FAP is expressed on some fraction of stromal fibroblasts in more than 90% of patients with solid tumors, and patients with higher FAP expression have a worse clinical outcome. Thus, as Kraman *et al.* suggest, targeting FAP-expressing stroma cells for destruction could unmask the patients' own or adoptively transferred immunity. But in which clinical settings could these results influence future immunotherapy of cancer?

Human cancers when first detected usually have an average diameter of at least 1 cm and contain about 10⁹ cancer cells, including thousands of diverse heritable variants resistant to drugs, radiation, and immunotherapy. Metastatic cells may already be widely dispersed. Kraman *et al.* treated relatively small tumors in mice soon after they were inoculated with

cancer cells. Eliminating FAP⁺ stromal fibroblasts should inhibit growth of small spontaneous tumors and thus may help eliminate clinically undetectable cancer cells that have already metastasized before excision of the primary tumor, a common cause of relapse. The caveat is that metastatic cancer cells are not necessarily in the milieu of inflammation caused by experimental cancer cell injection. Experimentally, tumors 1 cm in diameter or larger in mice are abolished by cancer-specific T cells that target not only cancer cells but also stromal cells that also present can-

cer cell antigens (13, 14). The elimination of cancer cell variants by the immune system is presumably due to “bystander killing” that is secondary to elimination of stroma (13, 14). Thus, immunotherapy treatment with both T cells that target cancer cells and an agent that targets FAP-expressing cells for destruction could increase the success of eliminating solid tumors and metastatic cells.

References

1. M. Kraman *et al.*, *Science* **330**, 827 (2010).
2. D. Liao, Y. Luo, D. Markowitz, R. Xiang, R. A. Reisfeld, *PLoS ONE* **4**, e7965 (2009).

3. Y. Wen *et al.*, *Cancer Sci.* **101**, 2325 (2010).
4. N. Erez *et al.*, *Cancer Cell* **17**, 135 (2010).
5. H. Y. Chang *et al.*, *PLoS Biol.* **2**, e7 (2004).
6. I. Kryczek, S. Wei, E. Keller, R. Liu, W. Zou, *Am. J. Physiol. Cell Physiol.* **292**, C987 (2007).
7. M. C. Poznansky *et al.*, *Nat. Med.* **6**, 543 (2000).
8. L. P. Seung, D. A. Rowley, P. Dubey, H. Schreiber, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6254 (1995).
9. L. A. Pekarek, B. A. Starr, A. Y. Toledano, H. Schreiber, *J. Exp. Med.* **181**, 435 (1995).
10. I. Petit *et al.*, *Nat. Immunol.* **3**, 687 (2002).
11. L. Yang *et al.*, *Cancer Cell* **13**, 23 (2008).
12. J. Niedermeyer *et al.*, *Mol. Cell. Biol.* **20**, 1089 (2000).
13. M. T. Spiotto *et al.*, *Nat. Med.* **10**, 294 (2004).
14. B. Zhang *et al.*, *J. Exp. Med.* **204**, 49 (2007).

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PHYSICS

Antimatter Atomic Physics

H. R. J. Walters

Positronium (Ps), the bound state of an electron and its antiparticle, the positron, is the lightest neutral atomic species. The atomic nucleus is replaced by a positron that has only 1/1836 the mass of a proton. One way to characterize Ps atoms is to study how they scatter off other atoms and molecules, and it would be reasonable to expect Ps scattering to be some kind of coherent combination of electron and positron scattering. On page 789 of this issue, Brawley *et al.* (1) show experimentally that for impact energies up to 250 eV (2), Ps scatters as if it were just a free electron moving at the same speed. This result implies that the positron's interaction with the target is somehow “cloaked.” Whether each component of the total scattering, for example, the ionization contribution, is cloaked, or whether cancellation effects are at work, remains an outstanding and substantial theoretical challenge.

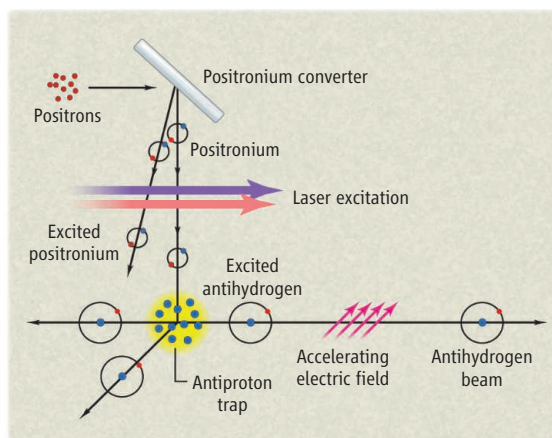
Positron interactions with atoms and molecules are difficult to treat theoretically because of the high degree of correlation (3). Theory has to describe how the light, agile positron competes with the slow, heavy atomic nuclei for the “attention” of the electrons in the system. Thus, it was only in 1997 that the first bound state of a positron with an atom was definitely established theoretically (4). The existence of such bound states between a positron and a molecule has subsequently been invoked to explain extremely high positron annihilation rates in certain molecular gases (5). The proposed

mechanism involves temporary capture of the positron by a molecule that is raised to an excited vibrational state. This state traps the positron for a sufficient length of time to markedly enhance its chance of annihilation by a molecular electron. Positron annihilation is the basis of medical positron emission tomography (PET) scanning. Because a positron will eventually be annihilated by an electron, even stable bound states have a short lifetime, on the order of 1 ns (10^{-9} s). Experimental observation of bound states is problematic. For example, it is only recently that the Ps₂ bound state (the analog of H₂),

predicted in 1947, has been observed (6).

The lifetime of bound systems depends on the alignment of the electron and positron spins. In Ps, there are two possible spin combinations, singlet and triplet, called para-Ps and ortho-Ps. The annihilation of an electron and positron in the lowest energy state of para-Ps predominantly forms two very high energy photons (γ -rays of 511 keV each) with a lifetime of 0.125 ns. Ortho-Ps is prevented by conservation laws from annihilating directly into two photons, and so annihilates predominantly into three photons with a lifetime of 142 ns. Only the ortho form of Ps is sufficiently long lived to be used in the beam experiments of Brawley *et al.*

These properties are relevant to an ambitious project to make a Bose-Einstein condensate of Ps (7), which would be the first example of a matter-antimatter condensate. The problem is to create a sufficiently dense gas of ortho-Ps in a microscopic cavity in a suitable material such as silica. The difficulty is that Ps-Ps collisions could convert ortho-Ps into para-Ps, which would then rapidly undergo destruction. However, if the electron and positron spins in all of the Ps atoms can be aligned in the same direction, this decay mechanism is not available. Recently, this type of spin alignment has been shown to be experimentally possible (8). If a condensate can



The positronium atom provides a gateway for making antihydrogen. In the proposed AEGIS experiment (13), Ps is to be created by bombarding a nanoporous material with positrons (Ps converter) and then laser-exciting it to a highly excited state. The excited Ps then exchanges its positron with a cold, trapped antiproton to produce highly excited antihydrogen, which is accelerated in an electric-field gradient to form a beam. ATRAP has shown the feasibility of a similar scheme. ATRAP and the former ATHENA collaboration have made antihydrogen by mixing positrons with antiprotons in a nested-well trap (9).

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be formed, then there is the exciting prospect of making a γ -ray laser (7) for probing objects on the nuclear length scale.

At the European Centre for Nuclear Research (CERN), there are four major collaborations (ALPHA, ATRAP, AEGIS, and ASACUSA) trying to make very slow (very “cold”) antihydrogen (9), the bound state of an antiproton and a positron (see the figure). The purpose is to test the fundamental CPT symmetry of relativistic quantum mechanics—inverting charge, parity, and time—and whether matter and antimatter behave in the same way in a gravitational field (10). There are major problems, such as how to make antihydrogen sufficiently cold, how to trap such a neutral species, and how to obtain it in its lowest energy state. At present, antihydrogen has been formed only in

very highly excited states.

One question is how long antihydrogen can be sustained in a trap before being destroyed in a collision with ordinary matter, such as traces of background gas, usually He or H₂. In such a collision, antihydrogen could be eliminated by one of many possible rearrangement processes (11). For example, the antiproton could be captured by a He⁺ ion, or by the antiproton annihilating with a proton in the nucleus of the background gas (12).

Early work in positronic atomic physics was hampered by the very low intensity of suitable positron sources. Experiments with Ps, such as in (1), are even more difficult in this respect. With improvements in intensity, positronic atomic physics should grow into an even more vibrant field.

References and Notes

1. S. J. Brawley *et al.*, *Science* **330**, 789 (2010).
2. One electron volt (eV) is the energy gained by an electron on being accelerated through a potential difference of one volt; a 250-eV Ps atom moves with a speed of just over 3 atomic units (au) in the dimensional units of atomic physics.
3. H. R. J. Walters, S. Sahoo, S. Gilmore, *Nucl. Instrum. Meth. B* **233**, 78 (2005).
4. G. G. Ryzhikh, J. Mitroy, *Phys. Rev. Lett.* **79**, 4124 (1997).
5. G. F. Gribakin, J. Young, C. Surko, *Rev. Mod. Phys.* **82**, 2557 (2010).
6. D. B. Cassidy, A. P. Mills Jr., *Nature* **449**, 195 (2007).
7. A. P. Mills Jr., *Nucl. Instrum. Meth. B* **192**, 107 (2002).
8. D. B. Cassidy, V. E. Meline, A. P. Mills Jr. *Phys. Rev. Lett.* **104**, 173401 (2010).
9. G. Gabrielse, *Phys. Today* **63**, 68 (2010).
10. M. Charlton *et al.*, *Phys. Rep.* **241**, 65 (1994).
11. E. A. G. Armour *et al.*, *Nucl. Instrum. Meth. B* **266**, 363 (2008).
12. E. A. G. Armour, M. Gregory, Y. Liu, *Nucl. Instrum. Meth. B* **247**, 123 (2006).
13. A. Kellerbauer *et al.*, *Nucl. Instrum. Meth. B* **266**, 351 (2008).

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PALEOCLIMATE

Increased Atmospheric CO₂ During the Middle Eocene

Paul N. Pearson

Even without humans, there are many processes that can change the concentration of carbon dioxide (CO₂) in Earth's atmosphere and affect global climate. On page 819 of this issue, Bijl *et al.* (1) provide the first direct evidence that very high CO₂ levels occurred about 40 million years ago during the Middle Eocene Climatic Optimum (MECO), one of the hottest intervals in Earth's climate history. The hunt is now on for a geological cause for this event—and fingers are pointing at the Himalayan mountain belt.

One measure of how fast this field is moving is that only 7 years have passed since the MECO was discovered (2). Geologists initially found its fingerprint in the oxygen isotope ratios of carbonate microfossils in drill cores from the Southern Ocean. These data indicated that the deep sea warmed by about 4°C, against a background climate that was already much warmer than today. Subsequent work on many other sites (3) confirmed that the warming was global, and now a rush of studies from all over the world is beginning to appear.

There are only three ways to cause a large and lasting increase in Earth's average surface

temperature: Turn up the heat from the Sun, reflect less sunlight back into space, or trap more heat in the atmosphere. The Sun is what astronomers call a “main sequence” star because of its age and chemical composition, and they expect it to maintain a nearly constant heat output over very long periods of time, so to argue that it might have increased its luminosity for an extended period without evidence seems gratuitous. Reducing Earth's reflectivity (albedo) can be accomplished in many ways—for example, by melting ice caps, by changing cloud types and cover, or by changing the land surface, such as by growing a forest where once there was a desert. Such effects, however, tend to follow from climate change rather than spontaneously causing it, and it is difficult to bring about such a major warming by this means alone. So from the beginning (2), researchers suspected that the MECO was due to a transient increase in atmospheric concentrations of greenhouse gases that trapped more heat, with CO₂ being the leading contender.

An ocean drill core confirms a dramatic rise in CO₂ levels 40 million years ago, just as the Himalayas grew.



Recycled. Destruction of carbonate rocks as the Himalayas formed might have contributed to a dramatic rise in atmospheric CO₂ 40 million years ago.

But how can researchers measure something as apparently fleeting as a trace atmospheric gas concentration from millions of years ago? The solution is to find something measurable in the sediment record that would have responded to the CO₂ level—a proxy. Bijl *et al.* use a relatively well-established method (4) to measure carbon isotope ratios in a group of organic compounds called alkenones, which are produced by a type of marine algae. This proxy is useful because the carbon isotope ratio is related to the dissolved CO₂ concentration in seawater, which in turn is in equilibrium with the atmosphere in most places. The method works well in more recent geological periods and in the modern ocean

(4), so it seems reasonable to apply it to the MECO, if enough alkenones can be found.

Bijl *et al.* focus on a core recovered from the East Tasman Plateau by the Ocean Drilling Program. Evidence of MECO warming was very clear in the core and alkenones were relatively abundant. The results are intriguing. Not only do they indicate a CO₂ peak coeval with the temperature rise, but the shapes of the reconstructed temperature and CO₂ profiles are broadly similar. They were able to calculate absolute CO₂ levels, indicating that the baseline level was already 1000 to 2000 parts per million by volume (ppmv) in the Eocene (versus 390 ppmv today and 270 ppmv before industrialization). Maximum levels in the MECO reached 4000 ppmv or higher—similar, perhaps, to a future anthropogenic greenhouse maximum. So the original idea (2) that the MECO was triggered by a transient CO₂ rise has survived a critical test.

If enhanced CO₂ caused the MECO, then these numbers can in principle be used to infer Earth's CO₂ "climate sensitivity" during the Eocene, that is, the global temperature change caused by a doubling of CO₂. Bijl *et al.* make a "tentative" attempt at this calculation—tentative because both the global temperature change and the CO₂ estimates are subject to a wide range of critical assumptions, the CO₂

proxy data are limited, the data are not entirely consistent with the temperature record in detail, and so far the evidence is from just a single site. Moreover, the proxy reconstructions have large error bars, especially for the top values. A more robust qualitative conclusion is that the CO₂ concentration was substantially higher than modern times, both before and after the event, and that it at least doubled during the MECO. Data from more sites, and other CO₂ proxies (5), are urgently required to flesh out the details.

The broad sweep of global climate history over millions of years has been described as one of "trends, rhythms, and aberrations" (6). The trends are long-term shifts in the mean climate state related to stately geological processes such as Earth's plate movements and the growth and decay of mountains. The rhythms are driven by cyclic variations in Earth's orbital parameters. Aberrations are sudden shocks caused by influences such as extraterrestrial impacts or violent volcanism, or they are threshold-crossing events. But the MECO does not sit comfortably within this classification. Its onset was too gradual to be an "aberration," and it was too long-lasting (~400,000 years). Even so, it was short-lived relative to the big trends we see in the geological record (6). Such features hint that the

MECO could yield new insights into the ways that geology forces Earth's climate system over long time scales.

The original hypothesis for the MECO (2) involved the disappearance of an ocean between India and Asia as the Himalayas were built. Did something unusual happen in this area about 40 million years ago that gradually released a huge amount of CO₂? One can imagine, for example, that the dying ocean, rich in carbonate sediments, was recycled through volcanic arcs and/or by extensive metamorphic decarbonation reactions. Bijl *et al.* have added to this debate by indicating how very large the CO₂ input might have been. The ball is now in the court of the Himalayan geologists: Is such a scenario still possible, or must we look elsewhere for the cause of the MECO greenhouse?

References

1. P. K. Bijl *et al.*, *Science* **330**, 819 (2010).
2. S. M. Bohaty, J. C. Zachos, *Geology* **31**, 1017 (2003).
3. S. M. Bohaty, J. C. Zachos, F. Florindo, M. L. Delaney, *Paleoceanography* **24**, PA2207 (2009).
4. M. Pagani, *Philos. Trans. R. Soc. London Ser. A* **360**, 609 (2002).
5. P. N. Pearson *et al.*, *Nature* **461**, 1110 (2009).
6. J. Zachos, M. Pagani, L. Sloan, E. Thomas, K. Billups, *Science* **292**, 686 (2001).

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NEUROSCIENCE

A New Viewpoint on Faces

Charles E. Connor

One of the most fundamental features of the primate brain is its organization into hierarchical networks for sequential information processing. The visual hierarchy is a densely interconnected network of more than 30 distinct brain regions. It radiates forward from the primary visual cortex near the back of the head along a dorsal (upper) "where" pathway (which processes space and motion) and a ventral (lower) "what" pathway (which processes objects and scenes) (1). Why are there so many stages of visual processing, and how do they function together to create visual experience and knowledge? Answering these questions has been especially difficult for the ventral pathway, due to the virtual infinity of potential objects the brain

can perceive. On page 845 of this issue (2), Freiwald and Tsao focus on one unique category of objects—human faces—as a paradigm for understanding hierarchical processing in the ventral pathway.

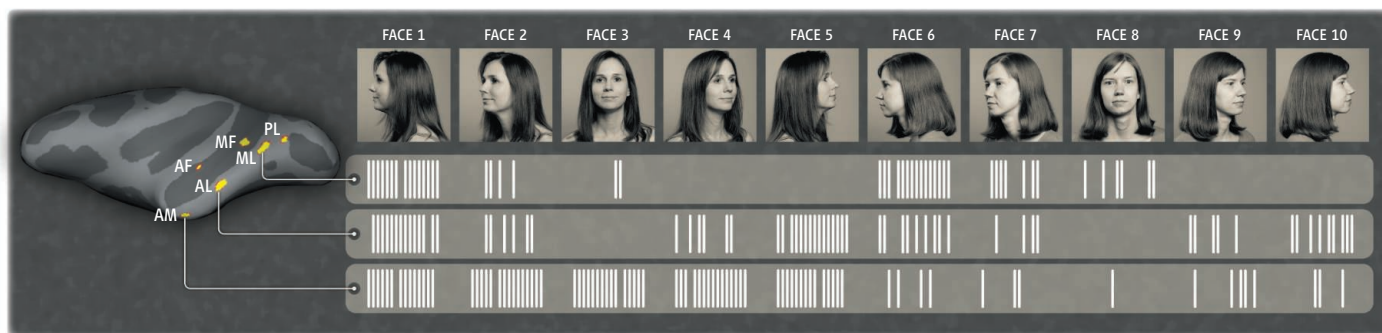
Faces are the most important category of objects for us and other primates, because they provide the principal basis for recognizing individuals, as well as a high-bandwidth channel for social communication. Faces have always been important to the study of the ventral visual pathway. The discovery of face-selective neurons in the monkey inferotemporal cortex (3), the highest, or most advanced, processing stage in the ventral pathway, initiated research into neural representation of complex objects. Faces have been used as a test case for studying fundamental questions about neural coding mechanisms (4), invariant representation (being able to recognize the same object from different viewpoints) (5), and neural processing dynamics (6). The

Part of the brain's visual hierarchy appears to be specialized for recognizing faces from any viewpoint.

discovery of the fusiform face area (7)—a ventral brain region involved in face perception—launched a new field focused on modular organization in the human visual cortex.

Faces have come to the fore again recently in a series of remarkable papers by Tsao, Freiwald, and their colleagues (8–10). These researchers have used a powerful combination of functional magnetic resonance imaging and targeted recording of neural signals in monkey brains to define a network of six interconnected face processing regions spanning the intermediate and higher-level stages of the ventral pathway. In their latest study, Freiwald and Tsao examine how the neural representation of individual identity and head orientation changes across three stages in the monkey brain. These experiments are a new approach to the crucial question of view-invariant recognition: How does the ventral pathway extract consistent information

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Face time. Neural selectivity for identity and viewpoint in the monkey face-processing network. Idealized neural spike trains exemplify response patterns at three stages in the network. Neurons in ML/MF respond to faces seen from a specific viewpoint,

regardless of identity. AL neurons show more selectivity for identity and generalize across mirror-symmetric viewpoints. AM neurons are strongly selective for identity, and generalize across many or all viewpoints. AF, anterior fundus; PL, posterior lateral.

about identity across multiple views of the same object?

Originally, investigators theorized that view-invariant object recognition is achieved by constructing view-invariant internal representations (11). More recent theories and experiments, however, argue that internal representations are view-dependent, meaning that invariant recognition is achieved by learning associations between views, based on their continuity in space and time (12, 13). Freiwald and Tsao provide a different perspective by comparing the selectivity of neural responses for viewpoints (e.g., profile) and identities (e.g., Amy versus Katy) across successive face-processing stages. The critical experiment involved recording neural responses to eight views (front, back, below, above, half-profiles, and profiles) of 25 humans (with whom the animals had no experience). The authors found that neurons in more posterior face-processing regions (middle lateral and middle fundus, labeled ML and MF, respectively, in the figure), are largely triggered by a single viewpoint (e.g., profile) and are relatively insensitive to individual identity (see the figure). Neurons in a more anterior region (anterior lateral or AL) showed more sensitivity to identity and a very specific kind of partial viewpoint invariance: selective responses to mirror-symmetric views (e.g., right and left profiles). Neurons in the most anterior region (anterior medial or AM) were most selective for identity and tended to generalize across many or all viewpoints.

These findings suggest that successive stages in the face network perform a step-wise transformation: from selectivity for viewpoint, regardless of identity, to selectivity for identity, regardless of viewpoint. The general implication is that earlier processing stages in the ventral pathway carry information about generic categories (e.g., face versus nonface) and viewpoint, whereas later processing stages carry information about individual exemplars (e.g., Amy ver-

sus Katy), eliminating viewpoint information to achieve invariant recognition. The caveat, of course, is that the special structure and behavioral role of faces may demand a unique processing strategy that does not generalize to other objects.

The new findings could be interpreted as a challenge to the idea that association between views is learned. A strict version of that idea would predict that recognizing Amy or Katy from different viewpoints depends on experiencing the close occurrence of those viewpoints in time and space. Freiwald and Tsao, however, present a clear case of view-invariant neural tuning that does not depend on specific learning. The animals no doubt became familiar with the photographs during the experiments, but the random presentation order provided no clues about which photographs shared the same individual identity.

The most striking aspect of the results is the mirror-symmetric viewpoint generalization in the AL region—for example, neurons that respond to right and left profiles but nothing in between. A more obvious intermediate step in a gradual invariance mechanism, especially learning by spatiotemporal association, would be for neurons to generalize across similar, neighboring viewpoints, for example, left profile and left half-profile. Instead, these AL neurons associate very different images that are related only through a complex geometric transformation (mirror reversal). This could represent a computational strategy that takes advantage of the fact that objects in our world frequently present mirror-symmetric views (14). Alternatively, the phenomenon might relate to the social importance of head orientation for primates. Frontal views signal social engagement and sometimes aggression, whereas profile views signal disengagement or appeasement. The conflation of right and left profiles at the neural level in AL might reflect their equivalent social

importance. There is an extensive literature showing how neurons located near AL, in the fundus and upper bank of the superior temporal sulcus, are sensitive to socially important variables such as head and gaze orientation and head and body movements (5). Thus, selectivity for viewpoint in ML, MF, and AL could reflect parallel processing of social information, as opposed to hierarchical processing of identity. In fact, given the opportunistic, co-optive nature of brain evolution, both interpretations are probably correct to some degree.

Freiwald and Tsao's study is bound to be the first of many exploiting the opportunity to study multiple stages in a specific hierarchical network. The results are certain to illuminate much more about hierarchical and parallel brain mechanisms for processing the most important object category—faces. Once again, the study of face perception seems poised to lead the way in understanding the ventral visual pathway.

References

1. D. J. Felleman, D. C. Van Essen, *Cereb. Cortex* **1**, 1 (1991).
2. W. A. Freiwald, D. Y. Tsao, *Science* **330**, 845 (2010).
3. C. Bruce, R. Desimone, C. G. Gross, *J. Neurophysiol.* **46**, 369 (1981).
4. D. A. Leopold, I. V. Bondar, M. A. Giese, *Nature* **442**, 572 (2006).
5. D. I. Perrett, J. K. Hietanen, M. W. Oram, P. J. Benson, E. T. Rolls, *Philos. Trans. R. Soc. London Ser. B* **335**, 23 (1992).
6. Y. Sugase, S. Yamane, S. Ueno, K. Kawano, *Nature* **400**, 869 (1999).
7. N. Kanwisher, J. McDermott, M. M. Chun, *J. Neurosci.* **17**, 4302 (1997).
8. D. Y. Tsao, W. A. Freiwald, R. B. Tootell, M. S. Livingstone, *Science* **311**, 670 (2006).
9. S. Moeller, W. A. Freiwald, D. Y. Tsao, *Science* **320**, 1355 (2008).
10. W. A. Freiwald, D. Y. Tsao, M. S. Livingstone, *Nat. Neurosci.* **12**, 1187 (2009).
11. D. Marr, H. K. Nishihara, *Proc. R. Soc. London B Biol. Sci.* **200**, 269 (1978).
12. T. Vetter, A. Hurlbert, T. Poggio, *Cereb. Cortex* **5**, 261 (1995).
13. N. Li, J. J. DiCarlo, *Science* **321**, 1502 (2008).
14. T. Vetter, T. Poggio, H. H. Bülthoff, *Curr. Biol.* **4**, 18 (1994).

10.1126/science.1198348

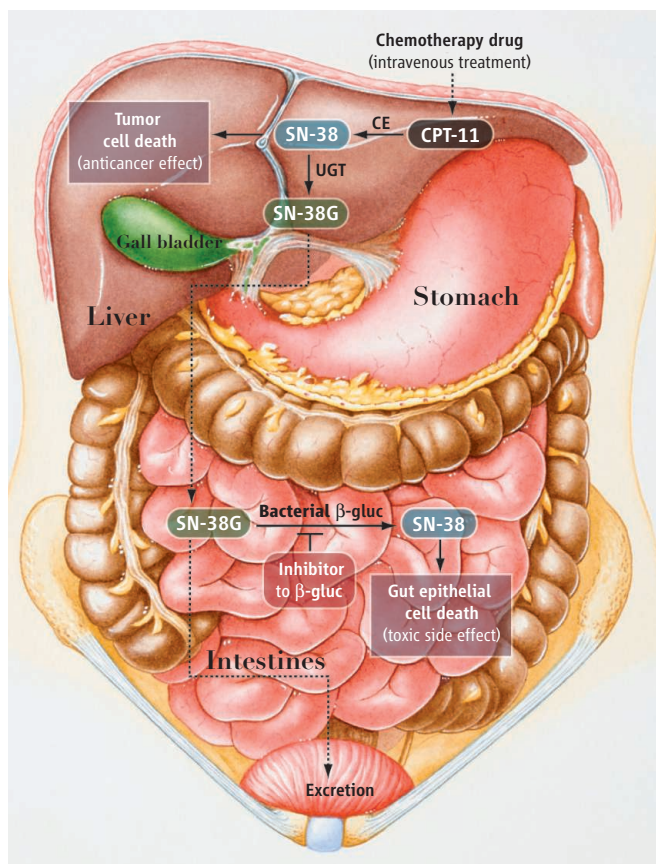
CANCER

Targeting Bacteria to Improve Cancer Therapy

Anand G. Patel¹ and Scott H. Kaufmann^{1,2}

The activity of many drugs is constrained by their metabolism. Agents that are administered as precursors (“prodrugs”) require enzymatic conversion to an active form; conversely, drugs given as active small molecules are converted to excretable entities or degraded by metabolic processes. In the clinic, this knowledge of drug metabolism has been exploited to enhance the efficacy of some drugs. On page 831 of this issue, Wallace *et al.* demonstrate that inhibiting an enzyme (β -glucuronidase) produced by good gut flora can prevent the intestinal metabolism of the anticancer drug irinotecan, thereby diminishing a life-threatening toxicity and conceivably allowing dose escalation that will enhance the drug's efficacy (1).

Irinotecan (also called CPT-11) is a water-soluble camptothecin analog that is active against metastatic colorectal carcinoma as well as cancers of the lung, pancreas, and breast. The drug's selectivity for cancer cells is attributed, in part, to the overexpression of its target, topoisomerase I, in cancer cells (2) and its ability to convert topoisomerase I into an agent that damages DNA (thereby killing the cell) (3). Irinotecan is also remarkable for its complex metabolism, which contributes to both its favorable pharmacokinetics and its unintentional adverse effects (see the figure). It is administered intravenously as a prodrug, which is converted by tissue and serum carboxylesterases into the active drug SN-38 (4, 5). SN-38, in turn, is converted by liver uridine diphosphoglucuronosyltransferases (UGTs) into SN-38-glucuronide (SN-38G), an inactive compound that is excreted through the biliary system into the gastrointestinal tract (4–6). Although SN-38G in the gastroin-



Good microbes, bad effect. Irinotecan (CPT-11) is administered intravenously as a prodrug. Carboxylesterases (CE) in various tissues convert CPT-11 into SN-38, which kills cancer cells. SN-38 can be inactivated in the liver by uridine diphosphoglucuronosyltransferase 1A1 (UGT), generating SN-38G that is excreted via bile into the intestine. Although SN-38G is not toxic to the intestinal mucosa, β -glucuronidases (β -gluc) produced by gut flora metabolize SN-38G to SN-38, which damages the gut mucosa. Inhibitors of β -glucuronidases prevent this conversion and diminish irinotecan gut toxicity in mice.

testinal tract is not toxic, β -glucuronidases expressed by intestinal commensal bacteria hydrolyze SN-38G to generate the toxic form, SN-38 (7).

It has been speculated that the metabolism of SN-38G to SN-38 in the gastrointestinal tract is responsible for severe diarrhea, a troublesome and dose-limiting toxicity of irinotecan (4, 5, 7). In an attempt to prevent this side effect, irinotecan has been administered with antibiotics (8, 9), but this has not gained widespread acceptance because eradication of beneficial gut microbes can cause malabsorption or allow colonization by pathogenic

Inhibiting an enzyme expressed by microbes in the human intestinal tract prevents an important toxic side effect of an anticancer drug.

bacteria not targeted by the antibiotic. An approach that selectively inhibits SN-38 reactivation in the gastrointestinal tract without killing the responsible bacteria might offer substantial benefit.

Wallace *et al.* screened 10,000 chemical compounds and identified four potent inhibitors of *Escherichia coli* β -glucuronidase. X-ray crystallography revealed contact between the inhibitors and a 17-residue loop structure that protects the active site of *E. coli* β -glucuronidase but is absent in the mammalian enzyme. When the authors generated a form lacking this loop, *E. coli* β -glucuronidase became insensitive to the inhibitors, consistent with their model.

The β -glucuronidase inhibitors also blocked enzymatic activity in intact *E. coli* and other bacteria, but they were not toxic to bacteria or cultured mammalian cells, suggesting the possibility of inhibiting SN-38G metabolism without harming bacteria or the gut. When Wallace *et al.* exposed mice to irinotecan with or without a β -glucuronidase inhibitor, the irinotecan-inhibitor combination resulted in much milder diarrhea. Histologic examination also showed that the severe damage to the gut mucosa was averted by this combined treatment.

Although the current emphasis in cancer chemotherapy is, quite appropriately, on the development of inhibitors that block oncogenic cellular signaling pathways, the findings of Wallace *et al.* serve as a reminder that some of the anticancer drugs already available can potentially be improved by incisive investigation. Much additional work, however, is required to bring this story to fruition. Further analysis is needed to refine the β -glucuronidase inhibitors identified in this study. Pharmacokinetic studies are needed to determine appropriate doses and treatment schedules for these compounds

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prior to actual testing in humans. In addition, extensive clinical testing is required to confirm that the β -glucuronidase inhibitors truly diminish irinotecan-induced gut toxicity and allow safe escalation of irinotecan doses in humans, either alone or in conjunction with cytokines to diminish the other major toxicity of irinotecan: myelosuppression (decreased production of white blood

cells). Nonetheless, Wallace *et al.* present an important first step toward improving the effectiveness of irinotecan chemotherapy.

References

1. B. D. Wallace *et al.*, *Science* **330**, 831 (2010).
2. I. Husain, J. L. Mohler, H. F. Seigler, J. M. Besterman, *Cancer Res.* **54**, 539 (1994).
3. Y. Pommier, *Nat. Rev. Cancer* **6**, 789 (2006).
4. R. H. J. Mathijssen *et al.*, *Clin. Cancer Res.* **7**, 2182 (2001).
5. A. Sparreboom, W. C. Zamboni, in *Cancer Chemotherapy & Biotherapy: Principles & Practice*, B. A. Chabner, D. L. Longo, Eds. (Lippincott Williams & Wilkins, Philadelphia, 2005), pp. 371–413.
6. S. Nagar, R. L. Blanchard, *Drug Metab. Rev.* **38**, 393 (2006).
7. K. Takasuna *et al.*, *Cancer Res.* **56**, 3752 (1996).
8. D. Flieger *et al.*, *Oncology* **72**, 10 (2007).
9. D. F. S. Kehrner *et al.*, *Clin. Cancer Res.* **7**, 1136 (2001).

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PLANT SCIENCE

Pollen Gets More Complex

Emily Indriolo and Daphne R. Goring

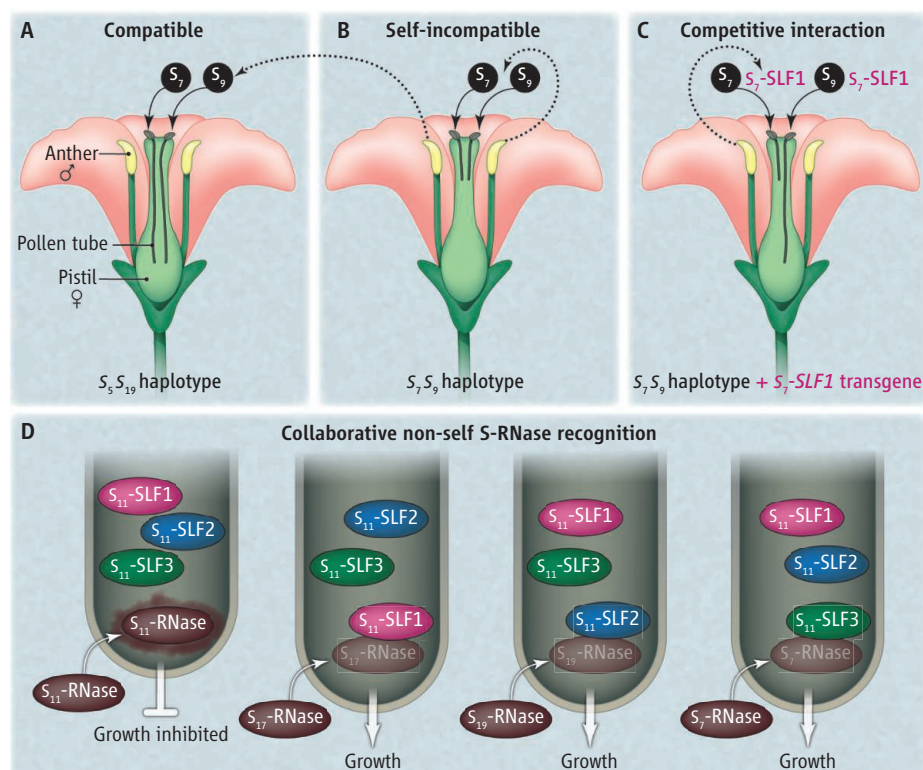
To prevent inbreeding, many flowering plants exhibit self-incompatibility (SI), a system for recognizing and rejecting their own pollen (1). In the Solanaceae, Rosaceae, and Plantaginaceae, the self-incompatibility trait is controlled by two physically linked *S* loci: the *S-RNase* gene representing the female *S* factor in the pistil and the *S-locus F-box* (*SLF*) gene representing the male *S* factor in the pollen (2–4). Both of these genes have many forms or alleles of *S-RNase* and *SLF*; a set of *S-RNase* and *SLF* alleles are inherited together as a single unit and are referred to as an *S* haplotype. The prevailing model for this system (see the figure) was that self pollen rejection occurs when self *S-RNases* enter the growing pollen tube and exert a cytotoxic effect, causing growth arrest. In contrast, *SLF* is required in compatible pollen tubes to block the action of non-self *S-RNases*, thus allowing for continued pollen tube growth and subsequent fertilization of the ovule. *SLF* is proposed to promote destruction of non-self *S-RNases* by the 26S-proteasome degradation pathway (2–4). However, there were some puzzling aspects of *SLF* that did not fully fit what would be expected for a pollen *S* factor, from both functional and evolutionary viewpoints (5). On page 796 of this issue, Kubo *et al.* (6) reveal that at least three related *SLF* genes encode pollen *S* factors in *Petunia* plants, and that these factors work collaboratively to prevent the pollen from being neutralized by non-self female cells. Their results represent a major shift in understanding how this SI system operates.

The prevailing model was useful, in part, because it explained a key process known as

competitive interaction (7) (see the figure). In this process, SI breaks down and self-fertilization can occur when the pollen genome contains more than one *S* haplotype. If two different *S* haplotypes were present in one pollen tube, then the male *SLF* proteins could suppress all the female *S-RNases*, and self-

In *Petunia*, collaboration among pollen genes enables cross-fertilization and prevents self-fertilization.

fertilization could occur. This model, however, posed some problems. From an evolutionary perspective, one tall order was that a single *SLF* gene had to encode a protein variant that could suppress all of the possible *S-RNase* variants, except for its self-*S-RNase*. So, for instance, the *SLF* variant *S*₇-*SLF*1



Working together. (A) Compatible pollination between distinct *S* haplotypes. The pollen *S*₇ and *S*₉ haplotypes are different from the *S*₅ and *S*₁₉ haplotypes present in the pistil, and so the pollen grains are accepted and pollen tube growth occurs. (B) Self-incompatibility. Pollen grains and pistil share the *S*₇ and *S*₉ haplotypes, and pollen tube growth is arrested. (C) Competitive interaction. The *S*₇-*SLF*1 transgene overcomes the pollen rejection response in the *S*₉-haplotype pollen. (D) Collaborative non-self *S-RNase* recognition model. Left panel: Self-pollination results in the self *S*₁₁-*RNase* entering the pollen tube and exerting a cytotoxic effect, as it is not detoxified by the *S*₁₁-*SLF*1, *S*₁₁-*SLF*2, or *S*₁₁-*SLF*3 proteins (the self-incompatibility response). The remaining three panels illustrate three different compatible cross-pollinations. Each non-self *S-RNase* is bound and detoxified by a different *S*₁₁-haplotype *SLF* protein, allowing for continued pollen tube growth.

would have to be able to suppress S_5 -RNase and S_9 -RNase but not S_7 -RNase. This one-to-many challenge was reinforced by phylogenetic analyses that found that the rate of change in the amino acid sequences of SLF and S-RNase allelic variants (synonymous substitution rates) were not equal; the non-self S-RNases appeared to be evolving more rapidly. This implied that *SLF* and *S-RNase* genes were not coevolving, unlike male and female gene pairs in other SI systems, such as the Brassicaceae (3, 4). Adding to the puzzle were observations that multiple pollen-expressed *SLF*-like genes were linked to the *S-RNase* gene (8–10), but it was unclear which one was playing the active role.

Kubo *et al.* solve some of these puzzles by revealing that there are at least three related *SLF* genes (renamed *SLF1*, *SLF2*, and *SLF3*) encoding pollen *S* factors that work collaboratively to suppress non-self S-RNases. In searching for other candidate pollen *S* genes, the authors analyzed 30 *SLF*-like sequences and grouped them into six types (*SLF1* through *SLF6*); all members of one type were highly similar to each other and were pre-

sumed to be allelic variants of that *SLF* gene. They found that the variation in the predicted amino acid sequence identities across the six types was much lower (~50%) than within a single type. This made evolutionary sense, because the pattern would allow for each SLF type to recognize a whole subset of non-self S-RNases. Finally, the researchers inserted allelic variants of the *SLF1*, *SLF2*, and *SLF3* genes into transgenic *Petunia* plants, looked for competitive interaction, and confirmed that each SLF type interacted only with a subset of S-RNases. The absence of competitive interaction in some plants implied that there are other potential SLF types involved in the suppression of the non-self S-RNases. So instead of a single SLF variant doing all the work, it is the combined actions of the allelic variants encoded by the different *SLF* genes that leads to the suppression of the non-self S-RNases (e.g., the S_{11} -*SLF1*, S_{11} -*SLF2*, and S_{11} -*SLF3* variants together can suppress the S_{17} , S_9 , and S_7 -RNases; see the figure). This allows for more non-self S-RNase variants to be inhibited, and increases the number of compatible mating partners for a particular plant.

It will be interesting to see whether this “collaborative” model also fits other S-RNase-based SI systems; tobacco, apple, and snapdragon species, for instance, also have multiple *SLF*-like genes linked to the *S-RNase* gene (8–10). A final question is, given the large number of *SLF* genes that have been found linked in the *S*-locus region, how many more of these SLF types are functioning in this collaborative recognition system?

References

1. V. E. Franklin-Tong, Ed., *Self-Incompatibility in Flowering Plants: Evolution, Diversity, and Mechanisms* (Springer-Verlag, Berlin, 2008).
2. Z. H. Hua, A. Fields, T. H. Kao, *Mol. Plant* **1**, 575 (2008).
3. B. J. McClure, *J. Exp. Bot.* **60**, 1069 (2009).
4. G. Chen *et al.*, *J. Exp. Bot.* **61**, 2027 (2010).
5. E. Newbigin, T. Paape, J. R. Kohn, *Plant Cell* **20**, 2286 (2008).
6. K.-i. Kubo *et al.*, *Science* **330**, 796 (2010).
7. J. F. Golz, H. Y. Oh, V. Su, M. Kusaba, E. Newbigin, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 15372 (2001).
8. J. Zhou *et al.*, *Sex. Plant Reprod.* **16**, 165 (2003).
9. D. Wheeler, E. Newbigin, *Genetics* **177**, 2171 (2007).
10. M. Minamikawa *et al.*, *Plant Mol. Biol.* **74**, 143 (2010).

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NEUROSCIENCE

Change in the Brain's White Matter

R. Douglas Fields

“Gray matter” is only one of two types of brain tissue; the other “white matter” is rarely mentioned. Yet white matter makes up half the human brain and has not been thought to be important in cognition or learning outside the context of pathology. That view could change. Imaging and cellular and molecular studies are revealing white matter plasticity with possible implications for normal cognitive function and psychological disorders.

White matter, which lies beneath the gray matter cortex, is composed of millions of bundles of axons (nerve fibers) that connect neurons in different brain regions into functional circuits. The white color derives from the electrical insulation (myelin) that coats axons (see the figure). It is formed by nonneuronal cells, oligodendrocytes, which wrap up to 150 layers of tightly compressed cell membrane around axons. Myelin is essential for high-speed transmission of

electrical impulses, and its damage can impair conduction and consequently, sensory, motor, and cognitive functions. The human brain continues to undergo myelination until at least the third decade of age, and the frontal regions of the cerebral cortex, which carry out higher-level executive functions, are the last to become myelinated.

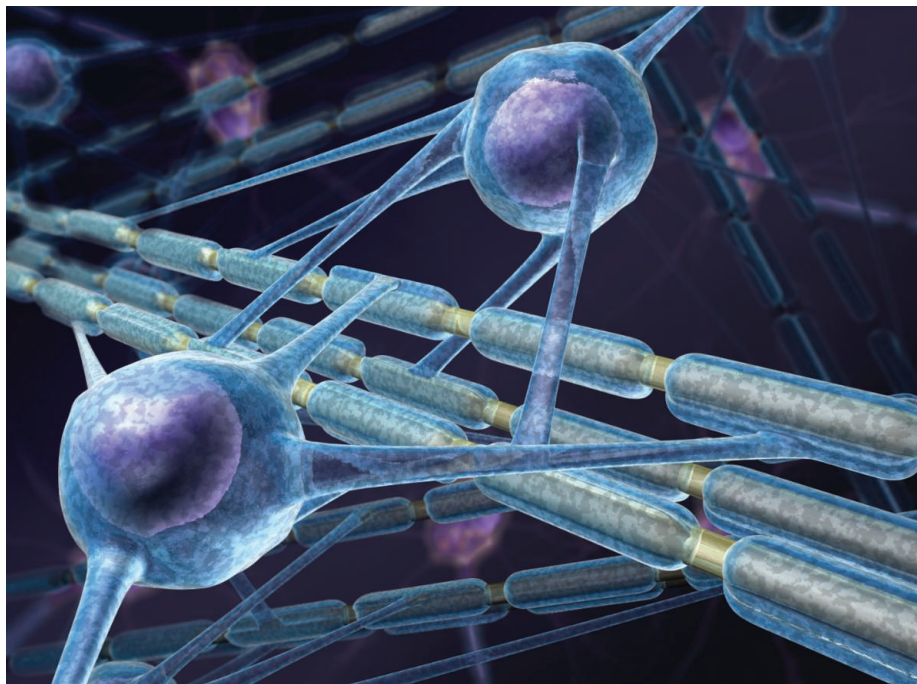
Learning involves changes in strength of synapses, the connections between neurons in gray matter. But human brain imaging using magnetic resonance imaging (MRI) has revealed structural changes in white matter after learning complex tasks. This raises the question of whether white matter responds to experience in a manner that affects neuron function under normal circumstances, thereby affecting information processing and performance. There are a few intriguing observations related to this possibility. For example, structural changes in white matter correlate with the number of hours a professional musician practices (1). The greatest changes were seen in parts of the brain that were not yet fully myelinated. Similarly, adult subjects showed

The role of the brain's white matter in active learning and memory may be underestimated.

increased white matter structural organization in a brain region important for visuo-motor control 6 week after learning to juggle (2). And in a study of adults learning to read, the volume, anatomical organization, and functional connectivity of white matter tracts linking cortical regions important for reading were increased (3). Whether these changes in white matter structure affect neuron function directly by altering transmission of information required for acquiring a skill is not clear. However, the observations do show that learning a new skill is associated with altered white matter structure in the mature brain.

Histological studies on experimental animals should clarify whether the white matter changes seen by MRI after learning are caused by myelination of unmyelinated axons, increased thickness of myelin on axons that are already myelinated, alterations in axon caliber, branching, or crossing, or other cellular changes. MRI analyses of Japanese macaques have shown, for example, large structural changes in white matter in the cerebellum after training them to

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White matter. Myelin that coats and insulates neuronal axons may control the propagation of electrical impulses in a manner that affects information processing.

use a rake to retrieve a food reward (4). The extent of change correlated with the speed of learning the skill. Studies on rats raised in enriched environments that provide social interaction and novel objects for exploration revealed robust cellular changes in gray and white matter that include vascular tissue, glia, neurons, and increased myelination (5). Whether myelin has a primary role in the increased information processing in such animal models should be further explored, as observations such as those in the rat-environment studies may have implications for understanding brain development during early childhood experience.

It is unclear whether experiences regulate myelination into adulthood. The size of the corpus callosum brain region increased by 10% in adult rats that were placed in an enriched environment for several months, but this was caused by an increased volume of another type of glial cell (astrocytes) as well as unmyelinated axons, possibly as a result of axon sprouting (6). The same treatment increased the volume of myelinated axons in the corpus callosum of juvenile animals. Thus, more axons appear to become myelinated as a result of experience during the developmental period when myelination is most active. Still, 29% of myelin-forming oligodendrocytes in adult mice develop from oligodendrocyte progenitor cells (OPCs) after sexual maturity (7). Perhaps this supply is generated for repair or possibly for myelination associated with learn-

ing. Interestingly, the cell division cycle of OPCs increases by 8 hours for every day of age from birth (8), indicating that the ability to form new oligodendrocytes decreases with age. This parallels the normal decline in human cognition and decrease in white matter volume after the age of 50 (9).

One of the largest categories of genes whose expression changes during sleep includes genes that control oligodendrocyte development and myelination (10). The reason for this is unclear, but sleep is linked to consolidating memory. Mutations in oligodendrocyte genes have been identified as possible risk factors for depression and schizophrenia (11), and disruption of specific genes in mouse oligodendrocytes correlates with behavioral changes resembling schizophrenia in humans (12). Mental disorders are currently understood to be disorders of synaptic transmission, but oligodendrocytes could perhaps contribute to aberrations in transmission.

How do oligodendrocytes know which axons are electrically active? Can impulse activity affect myelination? Three mechanisms have been identified that regulate myelination or the development of myelin-forming glia in response to electrical stimulation of axons in vitro. Specific frequencies of electrical impulses control the amount of L1 CAM present on unmyelinated axons, a cell adhesion molecule that is necessary for myelination (13). The neurotransmitter adenosine 5'-triphosphate (ATP) is

released from axons and activates receptors on astrocytes, causing them to release a cytokine (leukemia inhibitory factor) that stimulates myelination by mature oligodendrocytes (14). Adenosine derived from hydrolysis of released ATP promotes OPC development and thus increases myelination (15). Although synapses have been detected on OPCs, raising speculation that synaptic communication could stimulate myelination, neuron-OPC synapses are lost as OPCs mature to a premyelinating stage (16). Also, a nonsynaptic mechanism for ATP release from axons has been identified (17).

White matter is essential for impulse conduction, and so the concept of white matter plasticity widens the scope of investigation beyond the synapse in considering transmission of information through neural networks that are critical for learning complex skills and higher-level cognitive function in the absence of pathology. Perhaps white matter differences that correlate with scoring on intelligence quotient tests (18) and certain psychiatric conditions (11) can be attributed in part to a direct role for white matter in learning and cognitive function. But much work needs to be done to explore these interesting possibilities. This includes determining the nature of the white matter structural changes observed and assessing whether these changes affect electrical impulse transmission and/or synchrony of neuronal firing in a manner that affects information processing.

References

1. F. Ullén, *Neuron Glia Biol.* **5**, 29 (2009).
2. J. Scholz, M. C. Klein, T. E. Behrens, H. Johansen-Berg, *Nat. Neurosci.* **12**, 1370 (2009).
3. M. Carreiras et al., *Nature* **461**, 983 (2009).
4. M. M. Quallo et al., *Proc. Natl. Acad. Sci. U.S.A.* **106**, 18379 (2009).
5. J. A. Markham, W. T. Greenough, *Neuron Glia Biol.* **1**, 351 (2004).
6. J. A. Markham, M. M. Herting, A. E. Luszpjak, J. M. Juraska, W. T. Greenough, *Brain Res.* **1288**, 9 (2009).
7. L. E. Rivers et al., *Nat. Neurosci.* **11**, 1392 (2008).
8. K. Psachoulia, F. Jamen, K. M. Young, W. D. Richardson, *Neuron Glia Biol.* **5**, 57 (2009).
9. G. Bartzokis et al., *Neurobiol. Aging* **31**, 1554 (2010).
10. C. Cirelli, C. M. Gutierrez, G. Tononi, *Neuron* **41**, 35 (2004).
11. J. J. Lawrence, *Trends Neurosci.* **31**, 317 (2008).
12. K. Roy et al., *Proc. Natl. Acad. Sci. U.S.A.* **104**, 8131 (2007).
13. B. Stevens, S. Tanner, R. D. Fields, *J. Neurosci.* **18**, 9303 (1998).
14. T. Ishibashi et al., *Neuron* **49**, 823 (2006).
15. B. Stevens, S. Porta, L. L. Haak, V. Gallo, R. D. Fields, *Neuron* **36**, 855 (2002).
16. M. Kukley, A. Nishiyama, D. Dietrich, *J. Neurosci.* **30**, 8320 (2010).
17. R. D. Fields, Y. Ni, *Sci. Signal.* **3**, ra73 (2010).
18. V. J. Schmithorst, M. Wilke, B. J. Dardzinski, S. K. Holland, *Hum. Brain Mapp.* **26**, 139 (2005).

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Parental Control over the Brain

Christopher Gregg

Parents influence our brain development and behavior so substantially that they can set us on a course for life. Often we consider the impact of parenting styles or genetics in this context. However, maternally and paternally inherited chromosomes are not functionally equivalent, due to heritable epigenetic marks established in the parental gametes, called genomic imprints. Imprinting is thought to be rare in the genome, affecting ~100 genes in mice, and yet examples of transgenerational effects on gene expression, brain function, and the behavior of offspring are growing and increasingly mysterious. In one set of experiments, chimeric mice were generated by aggregating wild-type cells and cells containing either only maternal (parthenogenetic, PG) or paternal (androgenetic, AG) chromosomes. PG cells contributed preferentially to cortical and limbic brain regions, while AG cells contributed only to hypothalamic regions (1). From these findings, it was proposed that mothers and fathers preferentially influence the evolution and function of the cortex and hypothalamus, respectively. Several other parental effects have also been uncovered (2). In a study of genetically identical uniparental mice, a complex paternal transmission pattern of anxiety-related behaviors and growth effects was found that suggests epigenetic and sex-specific transgenera-

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Eppendorf and Science are pleased to present the prize-winning essay by Christopher Gregg, the 2010 winner of the Eppendorf and Science Prize for Neurobiology.

tional effects (3). Similarly complex effects have been described in humans (4).

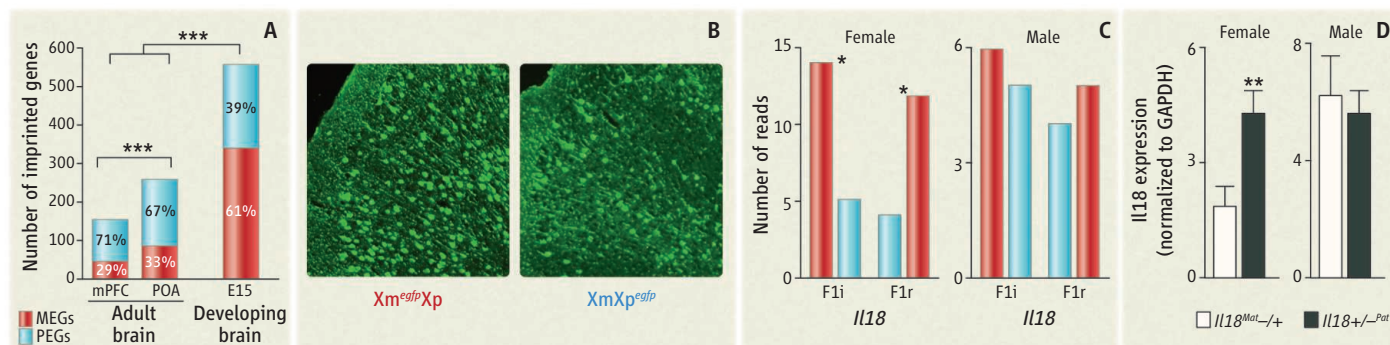
Parental effects have been clearly linked to human brain function and behavior through studies of Prader-Willi syndrome (PWS) and Angelman syndrome (AS), which result from a paternally or maternally inherited deletion of an imprinted gene cluster on chromosome 15, respectively. PWS is associated with hyperphagia, stubbornness, and compulsive traits (5), whereas AS is associated with absence of speech, a happy demeanor, and inappropriate laughter (6). Taken together, these studies highlight the potential for parental effects to influence the behavior and physiology of offspring, and suggest an underlying biology and epigenetic mode of inheritance that are clearly important but poorly understood.

My studies, with collaborators at Harvard, are focused on understanding the differences and functions of paternal and maternal gene expression programs in the developing and adult brain (7, 8). We initially mapped

Epigenetic marks on maternal and paternal genes differentially affect brain development in their children.

the expression pattern of 45 known imprinted genes across 118 adult brain regions. This study identified neural systems that are enriched for the expression of imprinted genes. We found the major monoaminergic nuclei of the brain (regions with serotonergic, dopaminergic, or noradrenergic neurons), as well as nuclei involved in feeding behavior, such as the arcuate nucleus, and social behavior, such as the preoptic area, to be enriched for imprinted gene expression. These initial observations prompted us to develop a genome-wide approach to study parental effects in specific brain regions at different developmental stages.

We first performed RNA-Seq analysis, a transcriptome profiling approach that uses deep-sequencing technologies, on cDNA libraries from two distantly related mouse strains, CASTeij and C57BL/6J, to identify all coding single-nucleotide polymorphisms (SNPs) that distinguish the two strains. We then performed RNA-Seq on specific brain regions of F₁ hybrid offspring generated by reciprocal crosses of CASTeij and C57BL/6J mice and used the SNP sites to distinguish expression levels from maternally versus paternally inherited alleles. Inspired by the chimera studies that suggested preferential maternal control over cortical regions and preferential paternal control over hypothalamic regions, we compared parent-specific gene expression programs in the adult medial prefrontal cortex (mPFC) and the preoptic area (POA) of the hypothalamus. The



Maternal and paternal gene expression programs in the brain. (A) Numbers of maternally expressed genes (MEGs) and paternally expressed genes (PEGs) in the adult and developing brain. **(B)** Preferential expression of the maternally inherited X (*Xm*) in the female mPFC revealed by crosses of an X-linked *egfp* reporter mouse to wild-type mice. **(C)** RNA-Seq analysis reveals preferential expression of the *interleukin-18* (*Il18*) maternal allele (red) relative to the pater-

nal allele (blue) in the female, but not male, mPFC (F1i, mouse strain CASTeij mother crossed with a C57BL/6J father; F1r, CASTeij father crossed with a C57BL/6J mother). **(D)** Quantitative polymerase chain reaction analysis of *Il18* expression in maternal versus paternal deletion *Il18* heterozygous mice reveals reduced expression in the mPFC of female, but not male, maternal deletion mice relative to paternal deletion mice.

2010 Grand Prize Winner

The author of the prize-winning essay, **Christopher Gregg**, received his B.Sc. in biochemistry from the University of Lethbridge, Canada. His graduate studies were carried out under Samuel Weiss at the University of Calgary. In 2006, he joined Catherine Dulac's laboratory at Harvard University as a postdoctoral fellow funded by the Alberta Heritage Foundation for Medical Research and the Human Frontiers Science Program. Gregg's postdoctoral work has focused on the development of next-generation sequencing approaches to study genomic imprinting and allele-specific gene expression programs in the brain. In 2011, he will join the University of Utah as an assistant professor, where he plans to work toward understanding genetic and epigenetic pathways that influence feeding and neuroeconomic decision-making processes.



University working with Jennifer Raymond and Richard Tsien on the molecular mechanisms of memory storage. In a collaboration with Georg Nagel and Karl Deisseroth, he pioneered the use of channelrhodopsin-2 for optical activation of neurons. His group at MIT has developed reagents for optically silencing neural activity and hardware platforms for light delivery into the brain, and has distributed these optogenetic tools to many groups worldwide.

Adam Kepecs, for his essay "Are you Certain? The Neural Basis for Decision Confidence." Kepecs received his bachelor's degree in computer science and mathematics at Eötvös Loránd University, Budapest, Hungary in 1997. He then switched to studying the brain, completing his Ph.D. in theoretical neuroscience in the laboratory of John Lisman at Brandeis University. In 2002, he joined the laboratory of Zachary Mainen at Cold Spring Harbor Laboratory, where he began studying decision-making in rats. Since



2007, he has been an assistant professor at Cold Spring Harbor Laboratory, where he uses quantitative behavioral models and electrophysiological, optical, and molecular techniques to study the neural circuitry underlying decision-making in rodents.

For the full text of finalist essays and for information about applying for next year's awards, see *Science* Online at www.sciencemag.org/feature/data/prizes/ependorf/.

Finalists



Ed Boyden, for his essay "Molecular Tools for Controlling Brain Circuits with Light." Boyden is an assistant professor at the Massachusetts Institute of Technology (MIT), where his group develops tools for controlling and observing neural circuits in order to understand how they compute and to yield new strategies for the treatment of brain disorders. He received his Ph.D. in neurosciences from Stanford

number of genes subject to parental effects in these regions was greater than expected (~372 genes) and involved complex isoform-specific parental effects. However, we did not find evidence for biased maternal control over the cortex. Instead, we found that in both the mPFC and POA, ~70% of autosomal genes exhibiting parental effects preferentially expressed the paternal allele (figure, panel A). Interestingly, an analysis of X-linked gene expression in females revealed preferential expression of the maternally inherited X in regions of the adult female brain, and this was confirmed with a transgenic approach (figure, panel B). In males, the X is strictly maternally derived. Previous work revealed that the X chromosome has evolved a preferential role in the regulation of the brain (9). We speculate that the autosomes and X chromosome give rise to paternal and maternal gene expression programs, respectively, which influence adult brain function and behavior.

Parental effects in the developing brain differed from those found in the adult. We found ~553 genes subject to parental effects in the embryonic day 15 (E15) brain, as compared to 257 in the adult POA and 153 in the adult mPFC (figure, panel A). Further, rather than

a paternal expression bias, 61% of the genes in the developing brain exhibited preferential expression of the maternal allele. These results reveal maternal effects that are specifically associated with brain development.

Finally, we analyzed males and females separately and uncovered evidence for sex-specific parental effects. An important example is *interleukin-18* (*Il-18*), which exhibits preferential expression in the female, but not male, mPFC (figure, panels C and D). *Il-18* has been linked to multiple sclerosis (10), a sexually dimorphic neurological disease that predominates in women and is associated with maternal parent-of-origin effects (11). In the POA of the hypothalamus, we also noted that females have three times the number of genes subject to sex-specific parental effects as males. The POA plays a central role in regulating maternal behavior. Given that maternal behavior alone affects offspring brain development and behavior, this result suggests a remarkable convergence of parental influences.

Our studies of parent-specific gene expression programs in the central nervous system suggest surprising and complex modes of parental influence over brain development and function in offspring. What are the

mechanisms that regulate these effects? How are maternal and paternal gene expression programs functionally related? Do parental influences on gene expression adapt to environmental pressures? How do these parental effects influence the behavior and physiology of offspring? What is the nature of parental effects in humans? These questions set a course for an exciting frontier and may shed new light on our understanding of brain evolution, function, and disease.

References

1. E. B. Keverne, *Prog. Brain. Res.* **133**, 279 (2001).
2. J. P. Curley, R. Mashoodh, *Dev. Psychobiol.* **52**, 312 (2010).
3. M. D. Alter et al., *Biol. Psychiatry* **66**, 1061 (2009).
4. G. Kaati, L. O. Bygren, M. Pembrey, M. Sjöström, *Eur. J. Hum. Genet.* **15**, 784 (2007).
5. S. B. Cassidy, D. J. Driscoll, *Eur. J. Hum. Genet.* **17**, 3 (2009).
6. G. Van Buggenhout, J. P. Fryns, *Eur. J. Hum. Genet.* **17**, 1367 (2009).
7. C. Gregg et al., *Science* **329**, 5992 (2010).
8. C. Gregg, Z. Jiangwen, J. E. Butler, D. Haig, C. Dulac, *Science* **329**, 5992 (2010).
9. DK. Nguyen, CM. Disteche, *Brain Res.* **1126**, 46 (2006).
10. S. Alboni, D. Cervia, S. Sugama, B. Conti, *J. Neuroinflamm.* **7**, 9 (2010).
11. I. A. Hoppenbrouwers et al., *Arch. Neurol.* **65**, 345 (2008).

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INTRODUCTION

Glee for Glia

RESEARCH ON GLIAL CELLS HAS COME OF AGE. UNTIL A FEW YEARS AGO, gliobiologists felt they had to justify their existence, often in a department of neuroscience or neurobiology, and they regularly stressed the abundance of glia as compared to that of neurons in the nervous system. Now, however, the outdated notion that glia are simply the glue that holds the nerve cells together but otherwise have no active role in the brain has been laid to rest for good. Years of thorough research and the steady accumulation of solid evidence have confirmed that glia are highly complex cells engaged in a plethora of functions. The role of glia in, among other examples, synapse formation, synapse maturation and plasticity, and the rapid conduction of action potentials, as well as their immunological functions in the nervous system, have by now been unequivocally established. Hence, this year's neuroscience special issue is devoted to glial cells.

Astrocytes are an important fraction of glial cells in the brain. The molecular mechanisms underlying astrocyte specification and growth and their interactions with other cell types to assemble the nervous system are still not completely understood. In his Review, Freeman (p. 774) presents and discusses exciting findings and ideas concerning the development, specification, and morphogenesis of astrocytes. Another type of glial cell, oligodendrocytes, is central to the brain's myelination, which ensures the efficiency and speed of action potentials. Emery (p. 779) reviews new approaches to investigating oligodendrocyte differentiation and myelination in the central nervous system. Microglia are another fascinating subpopulation of glial cells. They sense pathological tissue alterations and they can develop into brain macrophages and perform immunological functions. In a Perspective, Graeber (p. 783) outlines the involvement of microglia in the treatment of higher brain functional defects.

In the research section of the magazine, Lee *et al.* (p. 790) report that tonic inhibition in the cerebellum is due to GABA released from glial cells by permeation through the Bestrophin 1 anion channel. Ginhoux *et al.* (p. 841) investigate the developmental origin of microglia and show that adult microglia derive from primitive myeloid progenitor cells that arise before embryonic day 8. In a Perspective, Fields (p. 768) presents his hypothesis that white matter, which consists mostly of myelinated axons, may play a role in brain plasticity and learning.

The 9 November issue of *Science Signaling* complements this *Science* special issue on glia with research into signaling pathways involved in drug-resistant glioma, which is a type of aggressive brain cancer (www.sciencemag.org/special/glia). A Perspective by Ballanyi *et al.* describes how astrocytes sense changes in pH to stimulate the neurons that control breathing and, in a Podcast, Fields discusses mechanisms by which nerve and glial cells communicate outside of synapses.

— PETER STERN

Glia

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See also Perspectives p. 768; Research Article p. 790; Report p. 841; and Science Signaling at www.sciencemag.org/special/glia/.

Science

Specification and Morphogenesis of Astrocytes

Marc R. Freeman

Astrocytes are the most abundant cell type in the mammalian brain. Interest in astrocyte function has increased dramatically in recent years because of their newly discovered roles in synapse formation, maturation, efficacy, and plasticity. However, our understanding of astrocyte development has lagged behind that of other brain cell types. We do not know the molecular mechanism by which astrocytes are specified, how they grow to assume their complex morphologies, and how they interact with and sculpt developing neuronal circuits. Recent work has provided a basic understanding of how intrinsic and extrinsic mechanisms govern the production of astrocytes from precursor cells and the generation of astrocyte diversity. Moreover, new studies of astrocyte morphology have revealed that mature astrocytes are extraordinarily complex, interact with many thousands of synapses, and tile with other astrocytes to occupy unique spatial domains in the brain. A major challenge for the field is to understand how astrocytes talk to each other, and to neurons, during development to establish appropriate astrocytic and neuronal network architectures.

Neurons are not alone in the nervous system; the vast majority of all cells in the adult human brain are glia. Glial cell types in the CNS include astrocytes, oligodendrocytes, microglia, and chondroitin sulfate proteoglycan NG2-positive cells. Together these cells perform a dynamic range of functions essential for nervous system development and physiology, from simple trophic support of neurons to wrapping axons to allow for rapid nerve impulse conduction to modulating synaptic connectivity and efficacy. Astrocytes are the most abundant cell type in the brain; they are intimately associated with synapses and govern key steps in synapse formation and plasticity. However, we understand surprisingly little about the molecular underpinnings of astrocyte development. How are astrocytes specified at the appropriate developmental time from neural precursor cells (NPCs)? What gene expression profile distinguishes astrocytes from other glia or from neurons? How do newly born astrocytes interact with each other or with neurons to promote astrocyte maturation? This review will focus on recent advances in our understanding of how astrocytes are generated by NPCs and of astrocyte morphogenesis during nervous system assembly. Understanding these developmental events in molecular terms will provide insights into the mechanisms by which glia regulate brain development and function, as well as how changes in astrocyte function affect neurological disease.

Making Astrocytes—Nature and Nurture

During mammalian nervous system development, NPCs generate neurons first, followed by glia. Proceeding through the neuron-to-glia switch at the

proper time is critical for determining how many neurons or glia are ultimately made in each brain region. The mechanisms regulating this transition are complex and not well understood (1). However, a number of recent studies indicate that this fate switch is governed by both extrinsic environmental cues that promote astrogenesis in NPCs and NPC-intrinsic mechanisms that decrease neurogenic and increase astrogenic competence over developmental time.

Wnt signaling is required for the activation of the proneural genes *neurogenin1* (*ngn1*) and *neurogenin2* (*ngn2*) in NPCs, where, at an early stage, they act to promote neuronal differentiation (2–4). However, Wnt ligands continue to be expressed in the nervous system even after the neuron-to-glia transition, during which time they fail to induce *ngn* expression in NPCs. How are glia made when Wnts are still expressed? Hirabayashi *et al.* (5) found key intrinsic changes in histone H3 acetylation and trimethylation at the *ngn1* and *ngn2* promoters in NPCs, such that, at early stages, they observed high acetylation and low methylation, but at later stages, low acetylation and high methylation. These would correspond to the open or closed chromatin conformations, respectively. Treatment with histone deacetylase (HDAC) inhibitors resulted in an increase in *ngn* expression in older, but not younger, NPCs, which indicates that HDAC activity negatively regulates *ngn* expression in late NPCs. Consistent with a closed chromatin conformation at *ngn* loci, it was also observed that RNA polymerase II associated at relatively low rates with *ngn* promoters in late-stage NPCs. This epigenetic change in NPC competence is somehow mediated by the Polycomb group complex (PcG). Knockout of key components of the complex (e.g., *Ring1B* or *Ezh2*) prolongs the NPC neurogenic phase and delays the production of astrocytes (5). Thus, despite the continued presence of Wnts, NPCs are

able to make the neuron-to-glia switch in part because of intrinsic epigenetic changes that lead to PcG-dependent suppression of expression of the *ngns* (Fig. 1A).

Extrinsic signals also potently influence the fate of NPC progeny. This was demonstrated by culturing embryonic NPCs on either embryonic or postnatal cortical slices: NPCs made neurons when grown on embryonic slices, but glia when grown on postnatal slices (6). Thus, NPCs are competent to make astrocytes during embryonic stages, but normally do not, potentially because of the lack of some extracellular astrogenic cue. The Janus kinase–signal transducers and activators of transcription (JAK-STAT) pathway is a well-known activator of astrocyte cell fate and can be stimulated in NPCs by cytokines including ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and cardiotrophin-1 (CT-1) (1). Namiyama *et al.* used CT-1 to stimulate embryonic NPCs at either early or mid-gestational stages and found that CT-1 could promote astrocyte production only in mid-gestational or older NPCs. However, if Notch signaling was also activated in early NPCs, then treatment with CT-1 was sufficient to drive astrocyte production. How does Notch change NPC competence to respond to CT-1? Previous work had shown that methylation of the promoters of key astrocyte genes including *glial fibrillary acidic protein* (*gfap*) and *S100β* suppresses STAT binding and activation of these genes in NPCs. Moreover, blocking DNA methyltransferase activity in NPCs results in early induction of astrocyte genes (7). Thus, changes in DNA methylation status of astrogenic loci was a candidate mechanism. Expressing activated Notch (NICD) resulted in demethylation of STAT binding sites in the promoters and activation of expression of *gfap* and *S100β*. The mechanism by which Notch drives this event appears twofold: First, it promotes the dissociation of the DNMT1 maintenance methyltransferase [DNA (cytosine-5-)-methyltransferase 1] from the *gfap* promoter; second, it leads to increased nuclear factor IA (NFIA) expression (8). NFIA is a key regulator of the neuron-to-glia switch (9). NFIA is necessary and sufficient for induction of glial markers in NPCs like GLAST, is required for Notch to promote the transition to gliogenesis, and actively inhibits neurogenesis by binding to the *gfap* promoter and protecting it from DNMT1-dependent methylation (8, 9).

Positive Notch signaling is therefore sufficient to make NPCs prematurely responsive to CT-1 for activation of JAK-STAT signaling. But what drives normal activation of Notch in NPCs and, in turn, astrogenesis at the appropriate developmental time? The answer seems to be early-born neurons. Newly born *Ngn1*⁺ neurons, which are adjacent to NPCs, express the Notch ligands JAG1 and DLL1 and are thus a likely source for signals activating Notch signaling in NPCs (8). At the same time, CT-1 is secreted by embryonic neurons beginning at mid-gestation, which is thought to boost overall levels

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of CT-1 (10). It therefore appears that early-born neurons costimulate Notch and, through CT-1 release, JAK-STAT signaling pathways. Notch signaling shifts the chromatin state of NPCs to an astrogenesis-competent configuration, whereby CT-1 can activate astrocytic genes (Fig. 1B). These studies have revealed much about how NPCs shift from neurogenesis to gliogenesis and have led to many exciting new questions including: How are the initial epigenetic marks laid down so that NPCs make neurons, then glia, and not vice versa? What

promotes PcG-dependent repression of *ngn* loci over developmental time? Why doesn't early Notch signaling in NPCs (where it promotes maintenance of NPC fate) promote astrocyte production? Once Notch allows for STAT-dependent activation of astrocyte fate, what genes are turned on to promote astrocyte fate?

Astrocyte Diversity—Location Matters

Whether there is molecular and functional diversity among newly born astrocytes remains poorly

defined. In one (extreme) scenario, all newly born astrocytes could be essentially identical, with their mature phenotypes being shaped by interactions with their environment. Alternatively, astrocyte fates could be hard-wired, with astrocyte gene expression patterns and functional specializations that are largely predetermined at birth. Some intermediate scenario remains the most likely, as astrocytes are highly responsive to neuron-derived cues (below), and there is now good evidence that at least some aspects of vertebrate astrocyte fates are prepatterned. Positional identity along the dorsoventral (DV) and anteroposterior (AP) axis is a critical determinant of neuronal fates in the mammalian CNS. The ventricular zone is subdivided along the DV and AP axes by a combinatorial code of homeodomain transcription factors and precursors that generate unique pools of neuronal progeny that arise from distinct spatial expression domains (11). Recent work has identified three subpopulations of spinal cord white-matter astrocytes in the chick, which are organized along the dorsoventral axis (12). Each of these astrocyte populations is derived from a unique progenitor domain in the spinal cord (either VA1, VA2, or VA3 from dorsal to ventral) and has a unique pattern of expression of the markers Reelin and Slit1, and their fates appear to be regulated by homeodomain transcription factors which also govern diversification of neuronal fates (Fig. 2). For example, Pax6 is expressed in Reelin⁺ astrocytes, is required for Reelin expression, and, in the absence of Pax6, the border of Slit1 expression expands dorsally. Reciprocally, overexpression of Pax6 (even after the neurogenic phase is complete) is sufficient to expand the domain of Reelin expression ventrally and to suppress Slit1 expression (13). However, the functional significance of VA1, VA2, and VA3 white matter astrocyte diversity remains unclear. Moreover, these represent only a subpopulation of white matter astrocytes (which are themselves a subpopulation of all astrocytes), and it remains to be determined whether the homeodomain code governs the specification of additional populations of astrocytes in a similar way.

Beyond global DV-AP patterning mechanisms there is also evidence for more local regulation of astrocyte production from NPCs. The basic helix-loop-helix transcription factor stem cell leukemia (Scl) is required for distinguishing between astrocyte and oligodendrocyte fates, but only at the border of the pMN and p2 domains of the spinal cord and not in other regions of the spinal cord. This likely represents a patterning step (i.e., binary choice mechanism), rather than a mechanism for astrocyte specification, because Scl also modulates v2a versus v2b interneuron fates in the same region of the spinal cord (14). It is plausible that several such astrocyte subpopulations exist whose production from NPCs is modulated in a region-

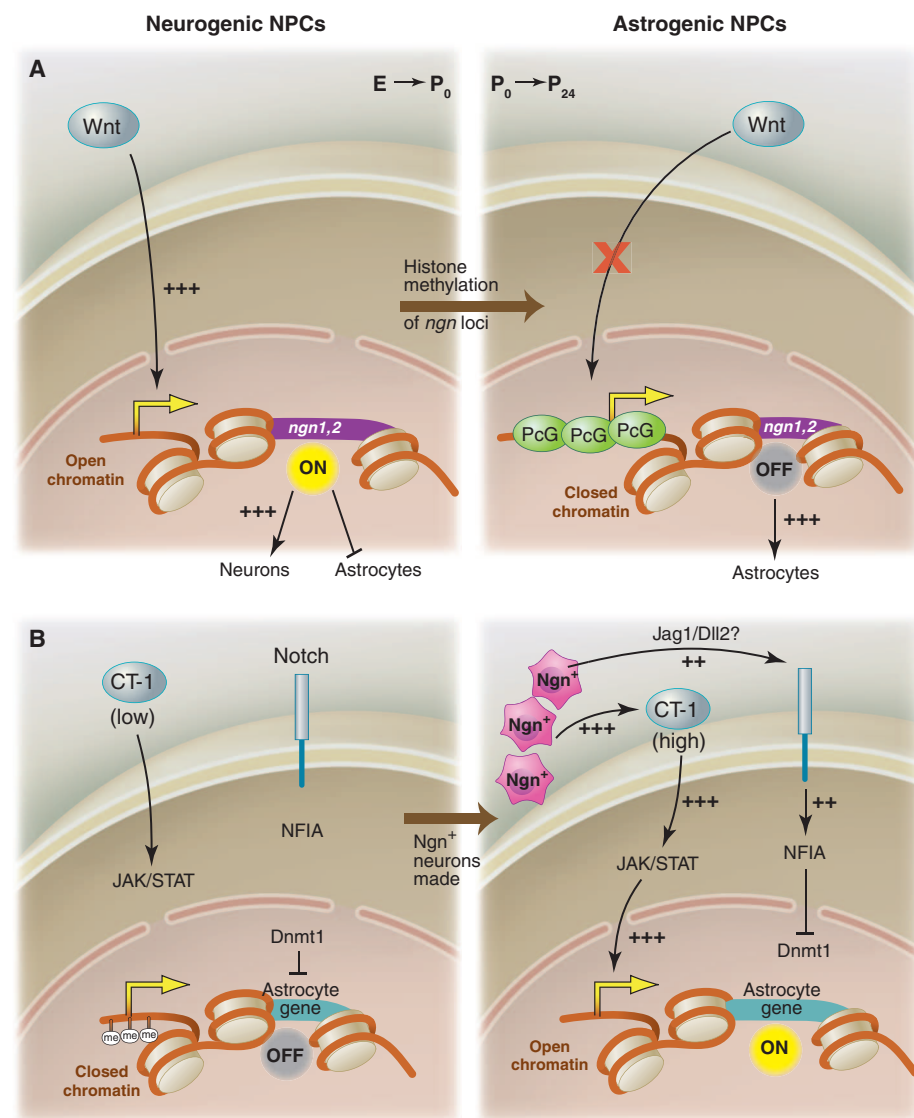


Fig. 1. Intrinsic epigenetic mechanisms converge with extrinsic signals to promote astrocyte fate from NPCs. (A) In early NPCs, chromatin is open at the *neurogenin1* and *neurogenin2* (*ngn1* and *2*) loci, Wnt signals can activate their expression, and neurogenins promote neuronal fate and inhibit astrocyte fate. In postnatal NPCs, the *ngn1* and *2* loci have been methylated and bound by PcG, chromatin is closed, Wnt-dependent *ngn1* and *2* expression is suppressed, and astrocyte fate is in turn derepressed. (B) Astrocyte genes are methylated during embryonic stages, which closes local chromatin; this is maintained by DNMT1, and astrocyte fate is suppressed, despite the presence of low levels of the proastrocyte factor CT-1. At the switch from NPC production of neurons to astrocytes, Ngn⁺ neurons release additional CT-1, which activates JAK-STAT signaling, and also activates Notch signaling (likely through JAG1 or DLL2), which activates NFIA, an inhibitor of Dnmt1 activity. Positive JAK-STAT signaling, coupled with opening of chromatin at astrocyte gene loci, promotes astrocyte fate.

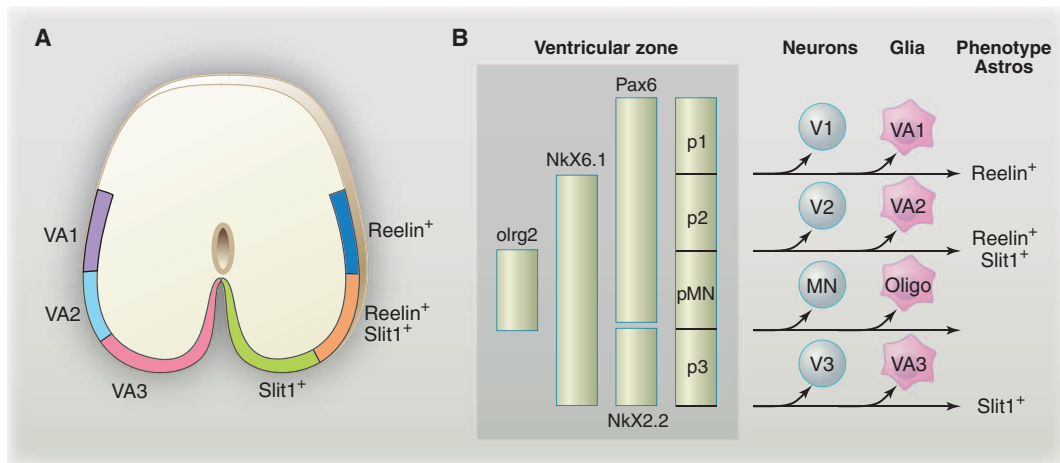


Fig. 2. Astrocyte diversity is generated by production from unique spatial domains. **(A)** A schematic illustrating VA1, VA2, and VA3 domains of the spinal cord white matter astrocytes and expression domains of Reelin and Slit1. **(B)** A homeodomain code that controls the generation of neuronal diversity is reused during astrocyte specification to promote white matter astrocyte diversity. [(A) and (B) are adapted from (12)]

ally restricted way, but such mechanisms await description. Positional identity is thus an important organizing feature of astrocyte populations, with mechanisms like homeodomain codes patterning globally and *Scl* ensuring production of subpopulations in binary choice scenarios. Because location matters, a model whereby astrocyte phenotype is primarily determined by astrocyte interactions with the environment seems increasingly unlikely.

The Black Box of Astrocyte Specification

Although the above-described pathways potentially modulate the neuron-to-glia switch in NPCs (i.e., when and where NPCs will make astrocytes), they do not seem to specify astrocyte fate. PcG genes act in the termination of neurogenesis (rather than induction of gliogenesis), JAK-STAT and Notch signaling makes NPCs competent to make astrocytes, and DV-AP patterning mechanisms determine where NPCs will make astrocytes.

COUP-TFI and II (chicken ovalbumin upstream promoter-transcription factors I and II) are also critical regulators of the induction of gliogenesis, but like the above-described pathways, they appear to regulate the timing of NPC competence to make glia (15), rather than specifying glial fates per se. Moreover, all of these pathways affect not only astrocyte development but also that of other glia subtypes (e.g., oligodendrocytes) and/or neurons. It should also be noted that these studies relied primarily on the activation of *GFAP* or *SL100β* as markers for astrocyte fates—these are known to be imperfect markers—and none traced these cells to

the point where they had acquired their mature morphology in the brain. As such, it remains unclear how astrocyte-like the cells of interest did, or did not, become. To date, no mutants exist where astrocytes are completely lacking, and no astrocyte-specific transcription factors have been identified. Thus, genes that direct an immature cell in the

called into question the classification of “glia” as a single brain cell class; expression analysis indicates that different glial subpopulations, such as astrocytes and oligodendrocytes, are as different from one another with respect to gene expression profiles as they are from neurons (17, 19). Both approaches have yielded treasure troves of interest-

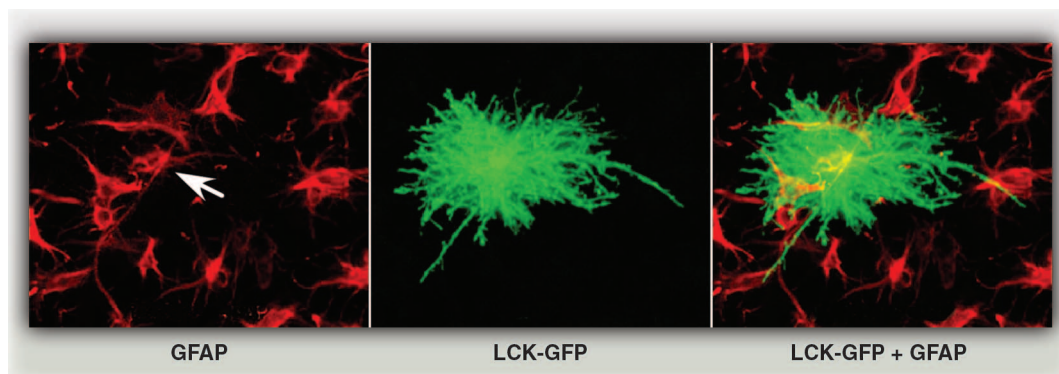


Fig. 3. Astrocyte morphology is far more complex than initially appreciated. Astrocytes in an organotypic slice preparation labeled with the common marker GFAP (red) overlaid with a single astrocyte transfected with the membrane marker Lck-GFP (green fluorescent protein). Arrow indicates GFAP label of Lck-GFP–marked astrocyte. [From (53) with permission from Elsevier]

nervous system to acquire the characteristics of a mature astrocyte remain to be discovered.

How will we move forward? The above study of DV-AP patterning of astrocytes was possible because Reelin and Slit1 allowed for the molecular discrimination of unique subsets of cells. New markers are needed to understand the development and diversity of these astrocyte subtypes. A recent large-scale screen of the spatial and temporal expression of more than 1400 mammalian transcription factors led to the identification of 12 with expression in spinal cord white matter in either oligodendrocyte or astrocyte lineages. Two of these

genes for future functional studies, but the next important steps include generating new markers to define the diversity and development of astrocyte populations, producing a new battery of astrocyte gene promoter-driven *Cre* lines to manipulate all or selected subsets of astrocytes, and ultimately defining which of these genes are key regulators of astrocyte specification, growth, and function.

The Mystery of Astrocyte Morphogenesis—Blossoms in the Brain

The morphology of a mature mammalian astrocyte is spectacular. From the cell soma radiate

genes, *Scl* and *Klf15*, were strongly enriched in expression in astrocytes, and their misexpression promoted precocious production of GFAP⁺ cells (16). A number of genomic approaches have been used to define the expression profiles of astrocytes and other major cell populations in the nervous system including GeneChip arrays (17, 18) and translating ribosome affinity purification (TRAP) (19). Comprehensive GeneChip arrays on highly purified astrocytes, oligodendrocytes, and neurons from multiple developmental stages provide a resource to explore how astrocytes change expression patterns over developmental time and have led to the identification of improved astrocyte markers like *Aldh1L1* (17). Interestingly, microarray and TRAP studies have both

primary branches that gradually divide into finer and finer processes to generate a dense network of delicate terminal processes, which associate very closely with synapses. Until recently, most studies used GFAP immunostains to characterize astrocyte morphology, but this marker only reveals the structure of primary branches, which represent a meager ~15% of the total volume of the astrocyte (20) (Fig. 3). Astrocytes are more morphologically complex than was initially appreciated. Impressively, depending upon the particular brain region, a single mature rodent astrocyte can cover a spatial domain in the brain that ranges between 20,000 and 80,000 μm^3 (20–22), wrap multiple neuronal somata (21), associate with 300 to 600 neuronal dendrites (21), and contact ~100,000 individual synapses (20, 23). In humans, these numbers increase dramatically, with a single astrocyte occupying a volume in the brain that is almost 30 times the volume in rodents and associating with ~2,000,000 synapses (23).

We know remarkably little about signaling pathways that direct immature astrocytes generated by NPCs to transform ultimately into mature astrocytes *in vivo*. Astrocytes sprout cellular processes as early as the first week of postnatal development, and most processes appear filopodial (i.e., actively growing) in nature. At this developmental time point, the borders of astrocytes are quite ragged, and long processes that extend well beyond the astrocyte's border are commonly observed. However, during weeks 3 and 4 of postnatal development, astrocyte processes ramify to an increasing degree, distal processes become much thinner and spongiform in morphology, and fine astrocytic processes densely infiltrate the brain tissue (24). Thus, astrocyte morphology appears to be “mature” by week 3 to 4. In two landmark studies using dye injections to differentially label neighboring astrocytes, it was shown that each astrocyte, in fact, occupies a unique spatial domain and forms discrete borders with neighboring astrocytes (20, 22). How these borders form remains an intriguing and unexplored question. Early in development in the hippocampus (at postnatal day 7), which corresponds to the active growth stage of astrocyte development, neighboring astrocytes exhibit significant overlap of processes. However, overlapping processes are “pruned” back, and discrete borders develop by postnatal day 14, and this resolution of spatial domains becomes even more evident by postnatal day 21

(24). It is now believed that astrocytes “tile” with one another, through a mechanism akin to dendritic tiling, to accomplish complete coverage of the brain space (Fig. 4, A and B). How these domains are established and whether they are predefined or generated stochastically remain to be determined. For example, do initially overlapping astrocytic processes simply retract, or are they, in fact, pruned by larger-scale degradative mechanisms (e.g., overlapping processes are severed, degrade, and are cleared from the CNS) as has been observed with pruned dendrites and axons (25)? Is the extreme gap junction coupling observed in mature astrocytes a mechanism for communication among astrocytes during the establishment or maintenance of these domains? Astrocytic domains have been proposed to function as “synaptic islands,” whereby a single astrocyte could coordinate the activity of all synapses within its unique spatial domain, but their functional relevance remains to be determined (21). The integrity of astrocytic domains is compromised in mouse models of epilepsy within a week after injury, with astrocytes exhibiting a dramatic decrease in fine processes and an increase in the overlap of thicker processes, and these changes persist for 6 months after injury (26). The breakdown of unique astrocytic domains may occur in an injury-specific manner in the CNS because reactive

astrocyte overlap was negligible when multiple brain regions were subjected to denervation (27).

What governs the branching and elaboration of astrocyte processes? Because astrocytes are sensitive to neuronal activity, it would be surprising if their morphology was not shaped during development in some way by local neural activity. Astrocyte membranes are extremely dynamic in live preparations of the mature brain and apparently more so near synapses than neuronal cell bodies (28), consistent with a correlation of motility with synaptic activity. In a number of physiological settings, astrocytes appear to respond morphologically to changes in neural activity (29, 30). For example, a wide range of studies have reported dynamic and activity-dependent changes in Ca^{2+} signaling in astrocytes (29); these include events that depend upon the major mammalian neurotransmitter glutamate (31, 32), and glial ensheathment of synapses appears to be increased after neuronal stimulation (33–35). Specific behaviors can also elicit coordinated Ca^{2+} signaling in glial networks *in vivo* (36). Astrocytes express an array of neurotransmitter receptors and transporters, as well as ion channels through which they likely sense neuronal activity and direct morphological changes (37, 38). However, the extent to which astrocyte growth during development

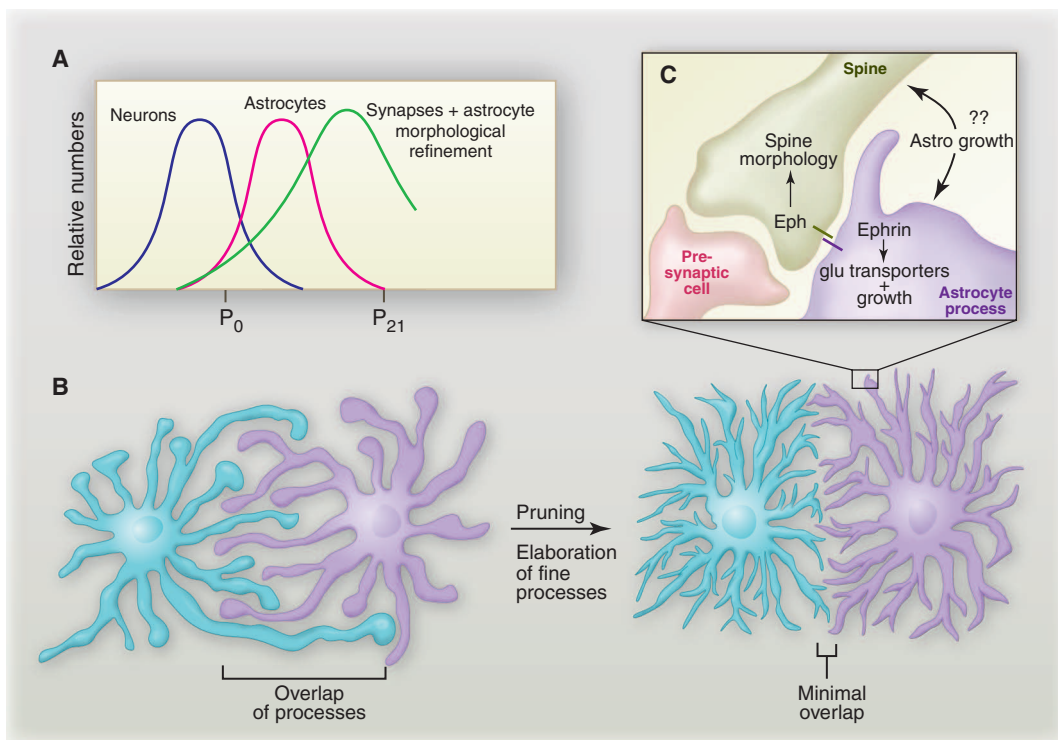


Fig. 4. Coordination of astrocyte morphological growth and refinement with synapse formation. **(A)** Although most neurons are made during embryonic stages, the major waves of synaptogenesis follow and depend on astrocyte production. The timing of astrocyte growth and morphological refinement overlaps significantly with this window of synaptogenesis. **(B)** Astrocytes initially extend large, filopodial processes that overlap significantly with neighboring astrocytes; however, by postnatal day 21, astrocytes refine their morphology to occupy unique spatial domains and elaborate fine processes that closely associate with synapses. **(C)** A complex interrelation exists between synapses and astrocyte processes. Eph or ephrin signaling can bidirectionally control astrocyte features, as well as spine morphology (see text).

depends on neuronal activity, or neuron-astrocyte signaling via other mechanisms, remains poorly defined.

Synaptogenesis and Astrocyte Maturation—Reciprocal Signaling During Brain Assembly?

How astrocyte growth is regulated such that initially simple cells can transform into a field of mature astrocytes with tiled and morphologically complex architecture remains a major question for the field. The close association of astrocytes with synapses, which is observed from even the earliest stages of development, is probably providing us with important clues. Astrocytes are now appreciated as important regulators of synapse formation, maintenance, efficacy, and elimination, but whereas increasing attention has been focused on how the synaptogenic activity of astrocytes sculpts neural connectivity, an equally interesting question is, do these newly formed synapses reciprocally shape astrocyte morphology? Not only is there is an intriguing coordinate timing between synapse formation and astrocyte birth (i.e., synapses form in earnest only after astrocytes appear), but the maturation of fine astrocytic processes and the establishment of astrocyte spatial domains seems to immediately follow the formation of new synapses (Fig. 4A). The major waves of rodent CNS synaptogenesis occur during the first 2 to 3 weeks of postnatal life (39–41). This is the time when astrocytes are dynamically growing. However, by weeks 3 to 4 (i.e., after the major wave of synapse formation), astrocytes have taken on their mature morphology, with fine processes closely associating with synapses and astrocytic spatial domains having been tiled out in the brain space. The expression of thrombospondins (TSP1 and TSP2), which are key astrocyte-secreted molecules that induce synapse formation, is high in the developing brain during week 1, but ceases by postnatal week 3 (42). This correlates with reduced synaptogenic potential of neurons and suggests that astrocytes down-regulate pathways that strongly promote synapse formation (41). Because astrocyte processes are broadly responsive to neural activity (30), it is easy to image how the initiation of synaptic activity could immediately begin to draw fine glial processes to newly formed synaptic contacts. For example, glutamate receptors (mGluRs) and transporters (EAAT1 and GLT-1) appear to be expressed in astrocytes during the first week of postnatal life (43, 44), which suggests that astrocytes are primed even at early stages to be responsive to neurotransmission. Accumulating evidence argues for an exciting and central role for Eph receptor–ephrin signaling in sculpting astrocyte-dendritic spine interactions. Astrocytic ephrin-A3 modulates dendritic spine morphology through neuronal EphA4, with activation of EphA4 negatively regulating spine growth (45), and there is an intriguing correlation between astrocyte contact and the maturation and lifetime of dendritic spines (46). Eph or ephrin signaling activates bidirectional

signaling between the engaged cells, which would offer an elegant mechanism for simultaneously coordinating astrocyte process growth and spine morphology through a single pathway. Indeed, EphA4 and/or ephrin-A3 can modulate expression of glutamate transporters in glia (47), which appears critical for synaptic plasticity (48) (Fig. 4C). However, the role of Eph or ephrin signaling in astrocyte growth control has only been explored in the mature brain in vivo, despite the fact that ephrins can stimulate filopodial outgrowth from astrocytes within minutes (49).

In Closing—It's Wide Open

The future looks very bright for the field of astrocyte biology. We have a new appreciation for the fact that astrocytes are far more than simple support cells. They are active participants in brain assembly and signaling, and it is abundantly clear that we have only scratched the surface of the depth of their biology. Interest in glial cells has been increasing dramatically over the past two decades, and this has led to the inevitable development of new sophisticated genetic tools with which to label and manipulate astrocytes in vivo. New tools that allow for temporally controlled deletion of genes, specifically in mouse astrocytes, along with improved high-resolution imaging techniques, are enabling researchers to address fundamental questions in astrocyte biology for the first time. These tools need to be expanded and exploited to pry deeply into astrocyte biology in vivo. There is also growing interest in the use of genetic model organisms like *Drosophila*, *Caenorhabditis elegans*, and zebrafish in the study of glial biology. *Drosophila* have glial subtypes that are morphologically and molecularly similar to mammalian astrocytes (50, 51). *C. elegans* glia share a range of developmental and functional properties with mammalian glia (52), and these organisms are amenable to rapid forward genetic screens. Now is the time to harness the powerful forward genetic approaches in these organisms to explore astrocyte development and function. From a developmental perspective, central questions for the field include the following: What gene expression profile and key genes make an astrocyte? How do astrocytes grow into intricate morphologies and associate with synapses? How dynamic is this association? Precisely how do astrocytes govern synaptogenesis or other aspects of neural circuit formation? How do synapses reciprocally govern astrocyte maturation? Finally, how are developmental mechanisms reused in the adult during plasticity or changed after injury or in disease?

References and Notes

1. F. D. Miller, A. S. Gauthier, *Neuron* **54**, 357 (2007).
2. Y. Hirabayashi et al., *Development* **131**, 2791 (2004).
3. N. Israsena, M. Hu, W. Fu, L. Kan, J. A. Kessler, *Dev. Biol.* **268**, 220 (2004).
4. C. J. Zhou, U. Borello, J. L. Rubenstein, S. J. Pleasure, *Neuroscience* **142**, 1119 (2006).
5. Y. Hirabayashi et al., *Neuron* **63**, 600 (2009).
6. T. Morrow, M. R. Song, A. Ghosh, *Development* **128**, 3585 (2001).

7. T. Takizawa et al., *Dev. Cell* **1**, 749 (2001).
8. M. Namihira et al., *Dev. Cell* **16**, 245 (2009).
9. B. Deneen et al., *Neuron* **52**, 953 (2006).
10. F. Barnabé-Heider et al., *Neuron* **48**, 253 (2005).
11. R. Shirasaki, S. L. Pfaff, *Annu. Rev. Neurosci.* **25**, 251 (2002).
12. C. Hochstim, B. Deneen, A. Lukaszewicz, Q. Zhou, D. J. Anderson, *Cell* **133**, 510 (2008).
13. M. Takahashi, N. Osumi, *Development* **129**, 1327 (2002).
14. Y. Muroyama, Y. Fujiwara, S. H. Orkin, D. H. Rowitch, *Nature* **438**, 360 (2005).
15. H. Naka, S. Nakamura, T. Shimazaki, H. Okano, *Nat. Neurosci.* **11**, 1014 (2008).
16. H. Fu et al., *J. Neurosci.* **29**, 11399 (2009).
17. J. D. Cahoy et al., *J. Neurosci.* **28**, 264 (2008).
18. D. Lovatt et al., *J. Neurosci.* **27**, 12255 (2007).
19. J. P. Doyle et al., *Cell* **135**, 749 (2008).
20. E. A. Bushong, M. E. Martone, Y. Z. Jones, M. H. Ellisman, *J. Neurosci.* **22**, 183 (2002).
21. M. M. Halassa, T. Fellin, H. Takano, J. H. Dong, P. G. Haydon, *J. Neurosci.* **27**, 6473 (2007).
22. K. Ogata, T. Kosaka, *Neuroscience* **113**, 221 (2002).
23. N. A. Oberheim, X. Wang, S. Goldman, M. Nedergaard, *Trends Neurosci.* **29**, 547 (2006).
24. E. A. Bushong, M. E. Martone, M. H. Ellisman, *Int. J. Dev. Neurosci.* **22**, 73 (2004).
25. L. Luo, D. O'Leary, *Annu. Rev. Neurosci.* **28**, 127 (2005).
26. N. A. Oberheim et al., *J. Neurosci.* **28**, 3264 (2008).
27. U. Wilhelmsson et al., *Proc. Natl. Acad. Sci. U.S.A.* **103**, 17513 (2006).
28. J. Hirrlinger, S. Hülsmann, F. Kirchhoff, *Eur. J. Neurosci.* **20**, 2235 (2004).
29. A. Nimmerjahn, *J. Physiol.* **587**, 1639 (2009).
30. D. T. Theodosis, D. A. Poulain, S. H. Oliet, *Physiol. Rev.* **88**, 983 (2008).
31. X. Wang et al., *Nat. Neurosci.* **9**, 816 (2006).
32. G. C. Petzold, D. F. Albeanu, T. F. Sato, V. N. Murthy, *Neuron* **58**, 897 (2008).
33. I. Lushnikova, G. Skibo, D. Muller, I. Nikonenko, *Hippocampus* **19**, 753 (2009).
34. J. Wenzel, G. Lammert, U. Meyer, M. Krug, *Brain Res.* **560**, 122 (1991).
35. C. Genoud et al., *PLoS Biol.* **4**, e343 (2006).
36. A. Nimmerjahn, E. A. Mukamel, M. J. Schnitzer, *Neuron* **62**, 400 (2009).
37. A. Verkhratsky, C. Steinhäuser, *Brain Res. Brain Res. Rev.* **32**, 380 (2000).
38. Y. Yang, J. D. Rothstein, *Astrocytes in (Patho)physiology of the Nervous System*, V. Parpura, P. G. Haydon, Eds. (Springer, Boston, 2009), pp. 69–105.
39. R. D. Lund, J. S. Lund, *Brain Res.* **42**, 1 (1972).
40. S. S. Warton, R. McCart, *Synapse* **3**, 136 (1989).
41. E. M. Ullian, K. S. Christopherson, B. A. Barres, *Glia* **47**, 209 (2004).
42. K. S. Christopherson et al., *Cell* **120**, 421 (2005).
43. M. R. Regan et al., *J. Neurosci.* **27**, 6607 (2007).
44. G. P. Schools, H. K. Kimelberg, *J. Neurosci. Res.* **58**, 533 (1999).
45. K. K. Murai, L. N. Nguyen, F. Irie, Y. Yamaguchi, E. B. Pasquale, *Nat. Neurosci.* **6**, 153 (2003).
46. H. Nishida, S. Okabe, *J. Neurosci.* **27**, 331 (2007).
47. M. A. Carmona, K. K. Murai, L. Wang, A. J. Roberts, E. B. Pasquale, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 12524 (2009).
48. A. Filosa et al., *Nat. Neurosci.* **12**, 1285 (2009).
49. M. W. Nestor, L. P. Mok, M. E. Tulapurkar, S. M. Thompson, *J. Neurosci.* **27**, 12817 (2007).
50. J. Doherty, M. A. Logan, O. E. Taşdemir, M. R. Freeman, *J. Neurosci.* **29**, 4768 (2009).
51. H. Kawai, N. Arata, H. Nakayasu, *Glia* **36**, 406 (2001).
52. S. Shaham, *Curr. Opin. Neurobiol.* **16**, 522 (2006).
53. A. M. Benediktsson, S. J. Schachtele, S. H. Green, M. E. Dailey, *J. Neurosci. Methods* **141**, 41 (2005).
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REVIEW

Regulation of Oligodendrocyte Differentiation and Myelination

Ben Emery

Despite the importance of myelin for the rapid conduction of action potentials, the molecular bases of oligodendrocyte differentiation and central nervous system (CNS) myelination are still incompletely understood. Recent results have greatly advanced this understanding, identifying new transcriptional regulators of myelin gene expression, elucidating vital roles for microRNAs in controlling myelination, and clarifying the extracellular signaling mechanisms that orchestrate the development of myelin. Studies have also demonstrated an unexpected level of plasticity of myelin in the adult CNS. These recent advances provide new insight into how remyelination may be stimulated in demyelinating disorders such as multiple sclerosis.

Within the vertebrate nervous system, the efficiency and speed of action potentials relies on myelin. Myelin is a specialized structure generated by glial cells, which extend compacted spirals of membrane around the axons of many neurons. Within the central nervous system (CNS), myelin is formed by oligodendrocytes. Developmentally, these cells are generated by subventricular cells in the brain and spinal cord that give rise to committed oligodendrocyte progenitor cells (OPCs) that divide and migrate throughout the CNS. These OPCs appear in successive waves; at early developmental stages they predominantly arise from ventral regions of the neural tube, but at later developmental stages these are largely replaced by dorsally derived OPCs (1). These OPCs can then terminally differentiate into postmitotic, premyelinating oligodendrocytes which, given the appropriate environmental cues, will further mature and myelinate nearby receptive axons.

That oligodendrocytes have a role in myelination has been appreciated for nearly a century. Penfield noted in 1924, for instance, "That these cells have to do with the formation and maintenance of the myelin sheath is born out by the facts...they are very numerous, especially in the white matter, and the position and relation of their cytoplasmic expansions to the myelin sheaths is similar to the arrangement of the sheath of Schwann" (2); astute observations given that myelin and the oligodendrocyte cell bodies could not be stained in the same sections by the methods of the time (Fig. 1). The importance of myelin for CNS functioning has long been apparent from human diseases such as multiple sclerosis (MS) and inherited leukodystrophies in which the integrity of the myelin sheath is lost, and from the severe phenotype of mutant mouse and rat strains in which the myelination process is disrupted. To date, most myelination research has been directed toward identifying mechanisms that

promote or inhibit it during development with the goal of developing strategies to promote repair in the demyelinated CNS. Myelin may exhibit substantial plasticity throughout adult life. This has sparked renewed interest in the myelination process given that this plasticity may have profound implications for neural functioning. Here, I present some of the major areas of research within the CNS myelination field and some recent discoveries about the biology of oligodendrocytes and their progenitors.

Extrinsic Signaling Mechanisms Controlling Oligodendrocyte Differentiation and Myelination

Perhaps not surprisingly given the importance of myelination for the proper functioning of the

CNS, the development of oligodendrocytes and myelination of individual axons is a highly regulated process controlled by a number of mechanisms. These include axonal surface ligands, secreted molecules, and axonal activity.

Extracellular ligands and secreted molecules. The simplest mechanism for determining whether an individual axon is myelinated would be the expression of inhibitory or permissive cues for myelination on the surface of the axon itself. This mechanism would also have the important benefit of allowing control of myelin at the subcellular level, explaining how individual axons proximal to an oligodendrocyte can be myelinated or unmyelinated rather than the oligodendrocyte indiscriminately myelinating the entire field of axons. Intriguingly, many of the axonally expressed ligands found to influence myelination to date have been inhibitory, preventing oligodendrocyte differentiation and/or myelination. These factors have included axonal expression of ligands such as Jagged, which signals via Notch in OPCs (3), PSA-NCAM (4), and LINGO-1 (5), all of which inhibit either OPC differentiation or myelination. In contrast to the peripheral nervous system in which axonal expression of neuregulins is the dominant permissive signal for myelination by Schwann cells, neuregulin signaling to oligodendrocytes is largely dispensable for myelination, though CNS overexpression of neuregulins does induce hypermyelination (6). This may be at least partially due to redundancies with other promyelination signals such as laminins (7), which can activate overlapping intracellular signaling pathways.

Signaling via the Wnt/ β -catenin pathway has emerged as a key regulator of oligodendrocyte development, though one with somewhat paradoxical roles. Wnt signaling via the canonical pathway is transiently activated in OPCs concurrent with the initiation of terminal differentiation. Both β -catenin activity and the expression of Tcf4/Tcf12 (a transcription factor that mediates the transcriptional effects of the Wnt/ β -Catenin pathway) are subsequently down-regulated in mature oligodendrocytes (8, 9). This down-regulation of Wnt signaling may be necessary for oligodendrocyte differentiation, as mutant mice with elevated Wnt/ β -catenin signaling in the oligodendrocyte lineage display blocked differentiation and hypomyelination (8). Paradoxically, however, deletion of the Wnt effector Tcf4 does not cause precocious oligodendrocyte differentiation as may be expected, but also blocks oligodendrocyte differentiation (10, 11). Wnt signaling may thus exert complex roles in myelination, acting in conjunction with Tcf4 to promote the initial stages of oligo-

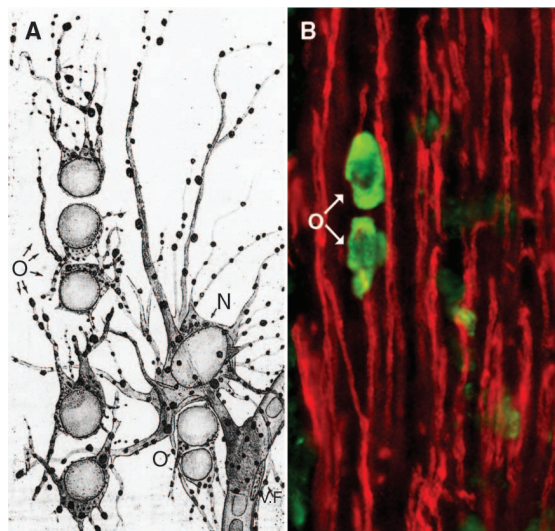


Fig. 1. (A) Drawing of silver-stained oligodendrocytes ("O") and a neuroglia/astrocyte ("N") by Penfield in 1924 (2). The myelin sheath was not stained in these preparations, thus the existence of a continual cytoplasmic link between oligodendrocytes and myelin was not demonstrated until the advent of electron microscopy. **(B)** Myelin sheaths in the developing mouse optic nerve stained with antibodies against myelin basic protein (red), with oligodendrocyte cell bodies stained with the β -catenin inhibitor adenomatous polyposis coli (green).

dendrocyte differentiation, but preventing subsequent differentiation steps and myelination unless down-regulated. These results have potential relevance for remyelination in human disease given that Wnt signaling components are present in MS lesions, suggesting that dysregulated Wnt/ β -catenin signaling could contribute to the lack of remyelination often seen in this disease (8).

In addition to the above factors, it is almost certain that a number of extracellular ligands that modulate CNS myelination remain to be identified. For instance, the orphan G protein-coupled receptor (GPCR) Gpr17 is transiently expressed during oligodendrocyte differentiation. Overexpression of Gpr17 causes severe dysmyelination, with oligodendrocyte differentiation stalling at an early stage; conversely, deletion of the gene results in precocious differentiation of OPCs into oligodendrocytes (12). The relevant ligand(s) for Gpr17 in this context have yet to be identified, but based on the phenotype of Gpr17 mutants they are presumably potent inhibitors of oligodendrocyte differentiation. Similarly, inhibition of γ -secretase activity within oligodendrocytes during their differentiation in neuronal co-cultures promotes formation of myelin segments (13). This effect is not mediated through obvious candidate γ -secretase substrates such as Notch, indicating that myelination can be inhibited through additional extracellular signals that act through a γ -secretase-dependent pathway. An orphan GPCR has recently been identified in zebrafish as having a vital role in promoting myelination by Schwann cells, raising the possibility that functionally equivalent GPCRs that promote myelination will be identified in oligodendrocytes (14). Proteomics and gene array studies that identify neuronally expressed ligands and oligodendrocyte lineage expressed receptors will likely be instrumental in identifying some of these currently uncharacterized promyelination signals.

Neuronal activity. In addition to genetically programmed extracellular ligands, there is evidence that myelination is at least in part driven by the level of electrical activity in the axons themselves (15, 16). This is particularly noteworthy because neural activity may also modulate ongoing myelination in the adult CNS, representing a form of neural plasticity (see below). There are a number of potential mechanisms by which this neuronal activity may promote myelination. Neuronal activity may modulate the surface expression of the abovementioned axonal ligands or cytokines,

though only limited evidence for such a mechanism exists at present (17). Alternatively, release of adenosine by active axons may activate purinergic receptors on OPCs and promote their differentiation and myelination (18). A less direct mechanism involves axonal release of ATP stimulating adjacent astrocytes to release the promyelination cytokine LIF, which in turn signals to oligodendrocytes (19).

Oligodendrocyte progenitor cells may be well equipped to receive synaptic input directly from neurons and respond accordingly. Direct stimulation of OPCs by glutamate released by synaptic-like

has a specific role in regulating OPC behavior. This suggests an elegant mechanism in which activity of unmyelinated axons is associated with direct synaptic release from axo-glial synaptic junctions onto adjacent OPCs, which differentiate and myelinate the axon at a certain signal threshold. However, hard experimental support for this concept is still lacking. Treatment of OPCs with glutamate *in vitro* can inhibit both their proliferation and subsequent differentiation via a block in rectifier K^+ channels (27); this suggests that the role of glutamatergic signaling to OPCs may be to limit, rather than promote,

myelination, possibly maintaining a pool of nondividing NG2-positive cells in the adult CNS. Moreover, although it has been reported that there are distinct populations of excitable and nonexcitable OPCs, with only the first group receiving synaptic input (25), it is not yet clear whether these groups display different capacities to myelinate and how they relate to the dividing and nondividing populations of OPCs also described in the mature CNS (28).

Intrinsic Control of Oligodendrocyte Differentiation and Myelination

It has long been appreciated that much of the regulation of oligodendrocyte behavior is intrinsic in nature, with mechanisms such as an internal "clock" limiting the number of cell divisions in OPC cultures grown in the absence of neurons (29). The past decade has seen great advances in our understanding of the nature of these intrinsic factors, which operate through transcriptional, posttranscriptional, and epigenetic mechanisms.

Transcriptional regulation. Chick electroporation and knockout mouse studies have proven instrumental in identifying a number of transcription factors required for the specification or differentiation of oligodendrocytes (Fig. 3). The initial specification of the oligodendrocyte lineage is reliant on the transcription factor Olig2; ventrally derived oligodendrocytes (and lower motor neurons) are derived from Olig2-expressing subventricular zone progenitors, and the oligodendrocyte lineage is absent in Olig2-null mice (30, 31). Subsequently, the downstream induction of a number of transcription factors, most notably Olig1, Ascl1, Nkx2.2, Sox10, YY1, and Tcf4, is required for the generation of mature, postmitotic oligodendrocytes (32). All these factors are present in OPCs as well as in postmitotic oligodendrocytes, with the exception of Tcf4, which is transiently expressed during differentiation (8, 11). This suggests that their roles

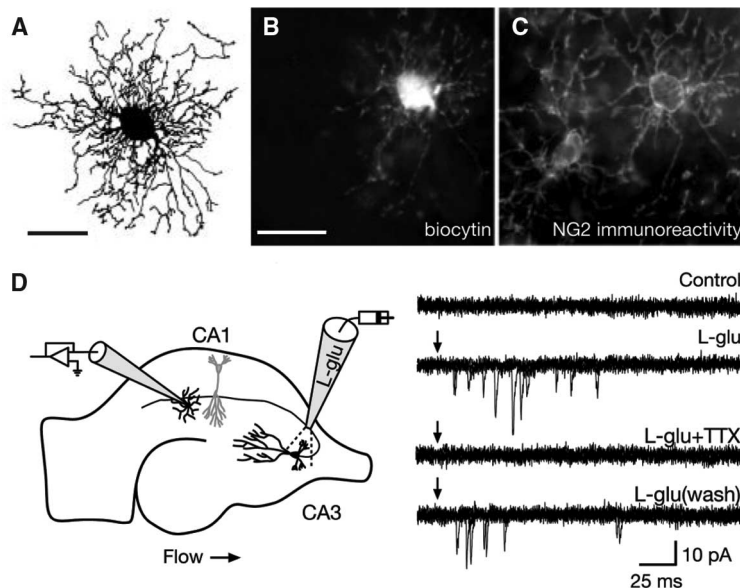


Fig. 2. Demonstration of OPC depolarization in response to synaptic input. [Reprinted by permission from Macmillan Publishers Ltd. (20)] (A to C) Example of one of the hippocampal OPCs recorded by Bergles *et al.* filled with biocytin (A and B) and stained with antibodies against the OPC marker NG2 (C). Scale bar, 20 μ m. (D) OPCs in the CA1 region were patch clamped and their membrane potentials measured in response to L-glutamate stimulation of CA3 neurons. Neuronal activity elicited bursts of inward currents in the OPCs (blocked by tetrodotoxin). The physiological role of these synaptic inputs to OPCs and the OPCs' ability to depolarize in response to them remains unknown.

structures was first described for the hippocampus (20) and has since also been observed in other gray and white matter regions and for the neurotransmitter γ -aminobutyric acid (GABA) (21, 22). Because OPCs express ionotropic glutamate receptors and voltage-gated ion channels (23), they can respond to this stimulation with a depolarization event not unlike the action potential of a neuron (Fig. 2). Although there is controversy over whether OPCs can generate bona fide action potentials and whether all OPCs can respond to synaptic input in this manner (21, 24, 25), the generation of miniature excitatory postsynaptic potentials by OPCs in response to glutamate stimulation has been a generally consistent finding. This synaptic input onto OPCs is also observed in the context of remyelination in the adult CNS (26) and is rapidly lost as the cells differentiate into mature oligodendrocytes (24, 26), suggesting that it likely

in promoting differentiation and activity at myelin gene promoters must be subject to regulation by additional factors differentially expressed between OPCs and myelinating cells. In this regard, a number of transcription factors, most notably Id2, Id4, Hes5, and Sox6, have been identified that are active in maintaining OPCs in their undifferentiated state and repressing myelin gene expression. This has led to a general “derepression” model of oligodendrocyte differentiation and myelination, whereby relief of extracellular inhibitory signals causes the down-regulation of these inhibitory factors or changes in their cellular localization, allowing pro-differentiation factors to induce differentiation and the expression of myelin genes.

The advent of wide-based gene expression analysis such as DNA microarrays has allowed for identification of many oligodendrocyte-specific or regulated genes with likely roles in regulating the myelination process (12, 33, 34). This enabled the identification of myelin gene regulatory factor (MRF), which is expressed within the CNS only by postmitotic oligodendrocytes, its expression being induced concurrent with terminal differentiation (33). Conditional inactivation of MRF within the oligodendrocyte lineage causes differentiation to stall at an early premyelinating stage, with the cells unable to express myelin genes or to myelinate. Conversely, forced expression of MRF within OPCs causes their precocious expression of myelin proteins (35). Given that the differentiation deficit seen in the absence of MRF appears broadly similar to that seen in the absence of factors such as Sox10 or Olig1, it is possible that cooperation with MRF upon its induction may allow these factors to have relatively specific roles in oligodendrocyte differentiation and myelin gene expression despite their earlier expression in OPCs. These findings add to the derepression model of oligodendrocyte differentiation by demonstrating that the transition from an OPC

into a myelinating oligodendrocyte requires the induction of promyelination factors, such as MRF, in addition to the down-regulation of inhibitory factors.

Chromatin remodeling. Oligodendrocyte differentiation is also regulated at the level of chromatin remodeling by histone deacetylases (HDACs), as pharmacological inhibition of HDAC activity in postnatal rats causes a delay in oligodendrocyte differentiation and myelination (36). Conditional deletion of HDAC1 and HDAC2 in the oligodendrocyte lineage causes a loss of both OPCs and oligodendrocytes (11), suggesting that this HDAC activity is required at multiple stages of the lineage. Generation of OPCs is retained in cortical progenitor-derived cultures from these conditional knockout mice; nevertheless, the differentiation of the OPCs into oligodendrocytes is still blocked, consistent with the *in vivo* pharmacological studies (36). Histone deacetylases likely promote oligodendrocyte differentiation by inhibiting the expression of pathways and genes that otherwise act to block differentiation. These include HDAC-mediated inhibition of the Wnt/ β -Catenin pathway (11) and HDACs acting in conjunction with the transcription factor YY1 to inhibit expression of factors such as Id4 and Tcf4 (37).

MicroRNAs. Posttranscriptional control of gene expression by microRNAs also plays a pivotal role in controlling CNS myelination. Conditional ablation of the Dicer enzyme (necessary for processing microRNAs into their active form) within the oligodendrocyte lineage in mice results in profound dysmyelination (38–40). Dicer (and by extension, microRNAs) is largely dispensable for the generation of OPCs in these mice; only the postmitotic stage of the lineage is severely disrupted. Consistent with this, the expression of Dicer itself increases during oligodendrocyte differentiation (33, 38, 39). Use of microRNA profiling identified several microRNAs, most notably miR-219 and

miR-338, that are induced concurrent with oligodendrocyte differentiation. These microRNAs target genes that usually act to maintain OPCs in the undifferentiated state, including PDGFR α , Sox6, and Hes5 (38, 39). This suggests a mechanism in which microRNAs form a positive-feedback loop during oligodendrocyte differentiation, such that key microRNAs induced early in differentiation act to inhibit the expression of genes that promote OPC maintenance, thus further inhibiting proliferation and promoting differentiation. More subtle but important roles have also been identified for microRNAs at other stages of the lineage. The miR-17-92 cluster regulates OPC proliferation via regulation of PTEN and thus Akt phosphorylation (41). Similarly, expression of microRNAs is required on a continual basis in mature oligodendrocytes for the proper maintenance of myelin, because conditional ablation of Dicer in mature oligodendrocytes causes a dysregulation of the expression of Elov17 and lipid homeostasis (40). These findings clearly demonstrate important roles for microRNAs in controlling CNS myelination at multiple stages.

Plasticity of Myelination in the Adult CNS

Although most myelination occurs early in life, myelination continues at least into late adolescence and, in some regions of the CNS, may increase throughout much of adult life (42, 43). In addition, there is evidence of plasticity of myelin in the adult CNS in response to changes in neural activity. Successful learning of juggling is associated with an increase in fractional anisotropy in the white matter underlying the intraparietal sulcus (a region of the brain involved in perceptual-motor coordination), suggesting an increase in myelination (44). Similar structural changes in white matter are associated with piano practice during childhood, and to a lesser extent, adulthood (45). The neuroimaging measures used in these studies are not especially

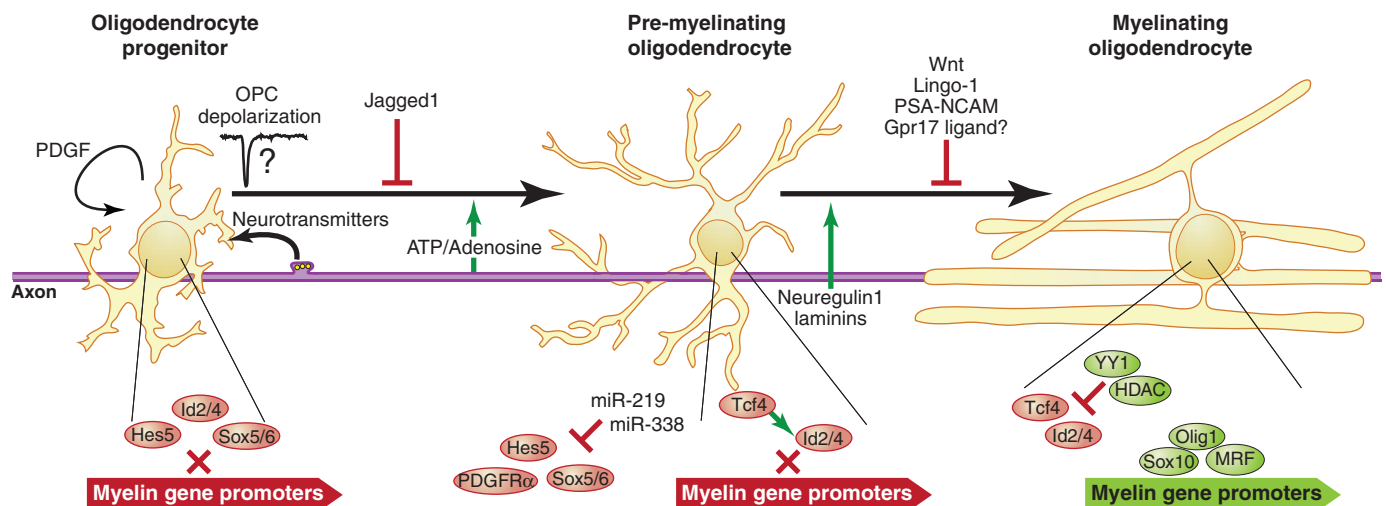


Fig. 3. Schematic of the oligodendrocyte lineage showing some of the intrinsic and extrinsic factors that influence oligodendrocyte differentiation and the myelination of individual axons. Oligodendrocyte differentiation requires the

integration of multiple extracellular signals through coordination of multiple intrinsic pathways. Myelination is regulated both at the level of oligodendrocyte differentiation and more subtly at the level of individual axons.

specific to myelination; other potential changes such as axonal diameter could contribute to the change in signal. Nevertheless, they do correlate well with previous findings in animal models that have documented increases in myelination after manipulations such as environmental enrichment (46). These findings have led to proposals that activity-related changes in CNS myelin could be considered a form of neural plasticity, whereby (presumably active) axons undergo myelination to improve the speed and efficiency of nerve conduction, thus strengthening or synchronizing specific connections (47).

This is still a largely uncharted area, and the real extent to which myelin plasticity may underlie forms of learning in the adult CNS is essentially untested. The question has profound implications both for normal learning and plasticity, and in light of findings of reduced myelination in psychiatric disorders such as bipolar disorder and schizophrenia (48). If aspects of learning in the adult CNS are mediated by ongoing myelination, an obvious question will be whether this adult myelination is regulated by the same mechanisms that drive myelination during development. Another open question is the source of new myelin in the adult CNS; is it generated by newly differentiated oligodendrocytes, or do mature oligodendrocytes display sufficient plasticity to respond to axonal signals and generate additional myelin segments? In support of the first hypothesis, there is a continuous differentiation of OPCs into myelinating oligodendrocytes in the adult CNS (28). The degree to which this ongoing differentiation is activity dependent is unknown; however, electrical stimulation of the corticospinal tract at the level of the hindbrain in the adult rat promotes the proliferation of OPCs within the spinal cord (49). At least some of these OPCs differentiate into postmitotic oligodendrocytes that closely appose the corticospinal axons, though whether they go on to myelinate the stimulated corticospinal tract axons preferentially over neighboring unstimulated axons is not clear.

Conclusions and Future Directions

The past decade has seen major advances in our understanding of how myelination in the CNS is regulated; the use of transgenic and knockout mice in particular has demonstrated clearcut roles for many ligands and transcription factors in the myelination process. More recently, substantial control of oligodendrocyte development by HDACs and microRNAs has also been demonstrated. Increasingly, there will be a need to synthesize these different elements of regulation into a single model. This will require a much better understanding of how these various levels of regulation interact; how the extracellular signals affect intracellular signaling pathways; and how these in turn influence the expression and activity of transcriptional regulators, epigenetic regulators, and microRNAs. Recent work in the peripheral nervous system has made elegant inroads into synthesizing some of these elements in Schwann cells, delineating clearcut pathways be-

tween neuregulin signaling, intracellular calcium concentrations, and subsequent activity of transcription factors at myelin gene promoters (50). Similar work in the CNS will be of vital importance.

Although we now know that neuronal activity mediates myelination, the exact mechanisms by which this occurs are largely unknown. Moreover, although the existence of synaptic input to OPCs and their depolarization in response were first described a decade ago and have been intensely debated and studied since, the functional importance of these phenomena remains unresolved. Increasingly sophisticated tools such as optogenetics (51) are now available to label and manipulate the activity of individual neurons in a tightly controlled manner. Such approaches should be able to determine whether modulation of activity in an individual axon can promote its myelination independently of its neighbors both during development and in the adult, and if so, by what mechanisms. Conversely, genetic approaches manipulating the expression of key receptors or voltage-gated ion channels specifically in OPC populations or manipulating the depolarization of these cells will clarify the role of synaptic inputs onto OPCs and the OPCs' ability to depolarize in response.

A major future challenge will be translating our knowledge of oligodendrocyte development and myelination into therapeutic approaches aimed at promoting remyelination in human diseases such as the leukodystrophies and MS. In early MS, remyelination can occur relatively robustly, but becomes less efficient with disease progression. Given that mature oligodendrocytes are relatively inefficient in initiating new myelin segments (13, 52), it seems likely that strategies promoting remyelination in such diseases will need to be targeted toward promoting the division, recruitment, and differentiation of OPCs and their subsequent myelination. It is not clear whether all mechanisms that regulate developmental myelination will have identical roles in remyelination; for example, unlike in development, Notch signaling does not appear to be a rate-limiting step in experimentally induced remyelination (53). Encouragingly, however, many of the mechanisms thus far identified as controlling developmental myelination do have conserved roles in remyelination. For instance, modulation of Lingo-1, known to regulate developmental myelination (5), also modulates remyelination in animal models of demyelination (54). Similarly, SVZ-derived OPCs receive synaptic input in the white matter in a mouse model of remyelination, indicating that, like developmental myelination, remyelination may be in part mediated by neuronal activity (26). We are still a long way from fully applying our understanding of mechanisms of myelin in a therapeutic context; however, the discoveries described here will provide an important basis for such work.

References and Notes

- W. D. Richardson, N. Kessaris, N. Pringle, *Nat. Rev. Neurosci.* **7**, 11 (2006).
- W. Penfield, *Brain* **47**, 430 (1924).
- S. Wang *et al.*, *Neuron* **21**, 63 (1998).
- P. Charles *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7585 (2000).
- S. Mi *et al.*, *Nat. Neurosci.* **8**, 745 (2005).
- B. G. Brinkmann *et al.*, *Neuron* **59**, 581 (2008).
- L. S. Laursen, C. W. Chan, C. French-Constant, *J. Neurosci.* **29**, 9174 (2009).
- S. P. Fancy *et al.*, *Genes Dev.* **23**, 1571 (2009).
- H. Fu *et al.*, *J. Neurosci.* **29**, 11399 (2009).
- H. Fu *et al.*, *Development* **129**, 681 (2002).
- F. Ye *et al.*, *Nat. Neurosci.* **12**, 829 (2009).
- Y. Chen *et al.*, *Nat. Neurosci.* **12**, 1398 (2009).
- T. A. Watkins, B. Emery, S. Mulinyawe, B. A. Barres, *Neuron* **60**, 555 (2008).
- K. R. Monk *et al.*, *Science* **325**, 1402 (2009).
- B. A. Barres, M. C. Raff, *Nature* **361**, 258 (1993).
- C. Demerens *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9887 (1996).
- K. Itoh, B. Stevens, M. Schachner, R. D. Fields, *Science* **270**, 1369 (1995).
- B. Stevens, S. Porta, L. L. Haak, V. Gallo, R. D. Fields, *Neuron* **36**, 855 (2002).
- T. Ishibashi *et al.*, *Neuron* **49**, 823 (2006).
- D. E. Bergles, J. D. Roberts, P. Somogyi, C. E. Jahr, *Nature* **405**, 187 (2000).
- S. C. Lin, D. E. Bergles, *J. Neurocytol.* **31**, 537 (2002).
- J. L. Ziskin, A. Nishiyama, M. Rubio, M. Fukaya, D. E. Bergles, *Nat. Neurosci.* **10**, 321 (2007).
- B. A. Barres, W. J. Koroshetz, K. J. Swartz, L. L. Chun, D. P. Corey, *Neuron* **4**, 507 (1990).
- L. M. De Biase, A. Nishiyama, D. E. Bergles, *J. Neurosci.* **30**, 3600 (2010).
- R. Kádár, N. B. Hamilton, Y. Bakiri, D. Attwell, *Nat. Neurosci.* **11**, 450 (2008).
- A. Etxeberria, J. M. Mangin, A. Aguirre, V. Gallo, *Nat. Neurosci.* **13**, 287 (2010).
- V. Gallo *et al.*, *J. Neurosci.* **16**, 2659 (1996).
- L. E. Rivers *et al.*, *Nat. Neurosci.* **11**, 1392 (2008).
- S. Temple, M. C. Raff, *Cell* **44**, 773 (1986).
- Q. Zhou, G. Choi, D. J. Anderson, *Neuron* **31**, 791 (2001).
- Q. R. Lu *et al.*, *Cell* **109**, 75 (2002).
- M. Wegner, *J. Mol. Neurosci.* **35**, 3 (2008).
- J. D. Cahoy *et al.*, *J. Neurosci.* **28**, 264 (2008).
- M. Heiman *et al.*, *Cell* **135**, 738 (2008).
- B. Emery *et al.*, *Cell* **138**, 172 (2009).
- S. Shen, J. Li, P. Casaccia-Bonnel, *J. Cell Biol.* **169**, 577 (2005).
- Y. He *et al.*, *Neuron* **55**, 217 (2007).
- X. Dugas *et al.*, *Neuron* **65**, 597 (2010).
- J. Zhao *et al.*, *Neuron* **65**, 612 (2010).
- D. Shin, J.-Y. Shin, M. T. McManus, L. J. Ptáček, Y.-H. Fu, *Ann. Neurol.* **66**, 843 (2009).
- H. Budde *et al.*, *Development* **137**, 2127 (2010).
- F. M. Benes, M. Turtle, Y. Khan, P. Farol, *Arch. Gen. Psychiatry* **51**, 477 (1994).
- T. Paus *et al.*, *Science* **283**, 1908 (1999).
- J. Scholz, M. C. Klein, T. E. Behrens, H. Johansen-Berg, *Nat. Neurosci.* **12**, 1370 (2009).
- S. L. Bengtsson *et al.*, *Nat. Neurosci.* **8**, 1148 (2005).
- J. M. Juraska, J. R. Kopcik, *Brain Res.* **450**, 1 (1988).
- R. D. Fields, *Neuroscientist* **11**, 528 (2005).
- D. Tkachev *et al.*, *Lancet* **362**, 798 (2003).
- Q. Li, M. Brus-Ramer, J. H. Martin, J. W. McDonald, *Neurosci. Lett.* **479**, 128 (2010).
- S. C. Kao *et al.*, *Science* **323**, 651 (2009).
- G. Miesenböck, *Science* **326**, 395 (2009).
- A. J. Crang, J. Gilson, W. F. Blakemore, *J. Neurocytol.* **27**, 541 (1998).
- M. F. Stidworthy *et al.*, *Brain* **127**, 1928 (2004).
- S. Mi *et al.*, *Nat. Med.* **13**, 1228 (2007).
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PERSPECTIVE

Changing Face of Microglia

Manuel B. Graeber

Microglia are resident brain cells that sense pathological tissue alterations. They can develop into brain macrophages and perform immunological functions. However, expression of immune proteins by microglia is not synonymous with inflammation, because these molecules can have central nervous system (CNS)-specific roles. Through their involvement in pain mechanisms, microglia also respond to external threats. Experimental studies support the idea that microglia have a role in the maintenance of synaptic integrity. Analogous to electricians, they are capable of removing defunct axon terminals, thereby helping neuronal connections to stay intact. Microglia in healthy CNS tissue do not qualify as macrophages, and their specific functions are beginning to be explored.

Microglia are less numerous than neuroglia but about as common as nerve cells. However, until 1991, a leading textbook of neuropathology stated (at the beginning of its chapter on microglia) that "... the microglia have become the most controversial element of the central nervous system; indeed, their very existence is in doubt," and the chapter concluded, "... the term 'microglia,' which implies a single distinct cell system, is misleading and no longer applicable" (1). Less than 20 years and more than 11,000 publications later, the microglia field has developed into a very active branch of neuroscience.

Microglial cells have an extremely plastic, chameleon-like phenotype (Fig. 1). This was demonstrated conclusively with the advent of lectin and antibody markers, which label all microglial activation stages and their successful application in experimental models such as the facial nucleus axotomy paradigm. As a result, the historical controversy surrounding the "nature and identity" of microglial cells that had lasted for decades was resolved, and the microglial cell type became firmly established. Before special stains were available, anatomists would see ramified microglia but could see "amoeboid" (macrophagelike) microglia only during central nervous system (CNS) development, whereas pathologists would encounter brain macrophages in different types of CNS lesions but rarely make the connection to ramified microglia as the source. The recent debate about seemingly contradictory neurotrophic and neurotoxic properties of microglia has to be viewed against the backdrop of microglial plasticity. Opposite biological effects of one and the same cell type are well established for macrophages (2).

Although the activity of microglia in immunological disease states (such as multiple sclerosis) and in the removal of myelin debris is in line with their pathological role as macrophages and antigen-presenting cells, the discovery of microglial involvement in neurogenesis, postlesional "synaptic stripping," and neuropathic pain under-

scores the existence of additional, functionally adapted microglial phenotypes. The present review attempts to highlight aspects of this emerging face of microglial cells.

The Microglial Immune Network Senses Threats to the CNS

In addition to their marked functional plasticity, microglial cells are characterized by a very low threshold of activation. They respond to even minor pathological challenges that affect the CNS (3), directly or indirectly. An early report on microglial activation detailed the detection of increased expression of complement receptors following a peripheral nerve lesion (4), demonstrating that a remote, sterile stimulus is a sufficient trigger for microglial activation. Microglial activation (5) occurs within minutes (6) but can be long-lasting. It is difficult to imagine any brain or spinal cord pathology without a microglial response. Based on this principle, an entirely new approach to cell type-specific nervous system imaging was developed by Banati *et al.* (see Fig. 2). However, the activation of microglia is anything but an unspecific process. The wide range of microglial response patterns and the great malleability of the microglial phenotype appear to be the result of the cells' ability to respond in a very graded manner to changes in their environment (Fig. 1) (7).

The microglial immune network has to be understood as a figurative system for catching or entrapping pathogens, because, in a strictly anatomical sense, microglial cells are not connected like neuroglia. Research has failed to demonstrate gap junctions between microglia *in vivo* at the ultrastructural level, although one study has suggested their existence under pathological conditions (8). In line with this anatomical constraint, the microglial response to lesions mirrors the location of an insult far more precisely than that of astrocytes, which, unlike microglia, establish a syncytial network. In other words, microglial cells are more individualistic and keep their distance from each other while covering their own surveillance territory. Because of this absence of direct intercellular coupling, microglial cell communica-

tion may have to rely more on auto- or paracrine mechanisms, as well as on purine and glutamate gradients (9).

The cell processes of normal microglia are mobile and scan their microenvironment, even in healthy state (10). Therefore, "resting" microglia are sessile, but they are not inactive cells (10). Consequently, the use of the term "resting microglia" without further explanation should be discouraged. Motility (that is, migration) of microglial cells is not usually observed in healthy CNS tissue, and the available literature suggests that when microglia become motile, considerable damage to CNS tissue has occurred, requiring structural and functional repair. In contrast, perivascular cells (11), which are resident in the Virchow-Robin spaces, can migrate and are constantly renewed from the bone marrow. Unlike most tissue macrophages, microglia have retained their proliferative potential. Perivascular cells constitutively express several molecules required for antigen presentation (11) and form an immunological blood-brain barrier. In contrast, microglia respond to threats to the CNS parenchyma proper and only express molecules such as major histocompatibility complex (MHC) antigens on demand. The expression of MHC class II molecules in human neurodegenerative diseases, which might be perceived as a nonspecific microglial reaction because it is so common, may in reality represent an important and, therefore, conserved mechanism to protect brain tissue that is already at risk (12). This view is supported by the intriguing finding that a human-specific gene expressed in cortical microglia, *SIGLEC11* (13), serves to mediate immunosuppressive signals and inhibits the function of microglial pattern-recognition receptors (14). The underlying human-specific gene conversion event has been said to be related to the evolution of the genus *Homo* (13), in keeping with the uniquely human evolution of sialic acid biology (15). Activation of the brain's microglial cells during systemic disease can provide an explanation for the feeling of illness and associated sickness behavior (16).

Activation of Microglia Can Be Painful

The role of microglia in the initiation of neuropathic pain appears to be crucial (17, 18). Neuropathic pain that occurs after peripheral nerve injury depends on the hyperexcitability of neurons in the dorsal horn of the spinal cord (18). At least five paths seem to exist that can lead to microglial activation in neuropathic nociceptive states, and the relevant molecular pathways include fractalkine, interferon- γ , monocyte chemoattractant protein-1, TLR4, and P2X4 as the main signaling mediator and/or receptor (17). In addition, microglial P2X7Rs and their downstream signaling pathways play a pivotal role in the induction of spinal long-term potentiation (LTP) and persistent pain induced by tetanic stimulation, which produces LTP of C-fiber-evoked field potentials in the spinal cord (19). Activation of

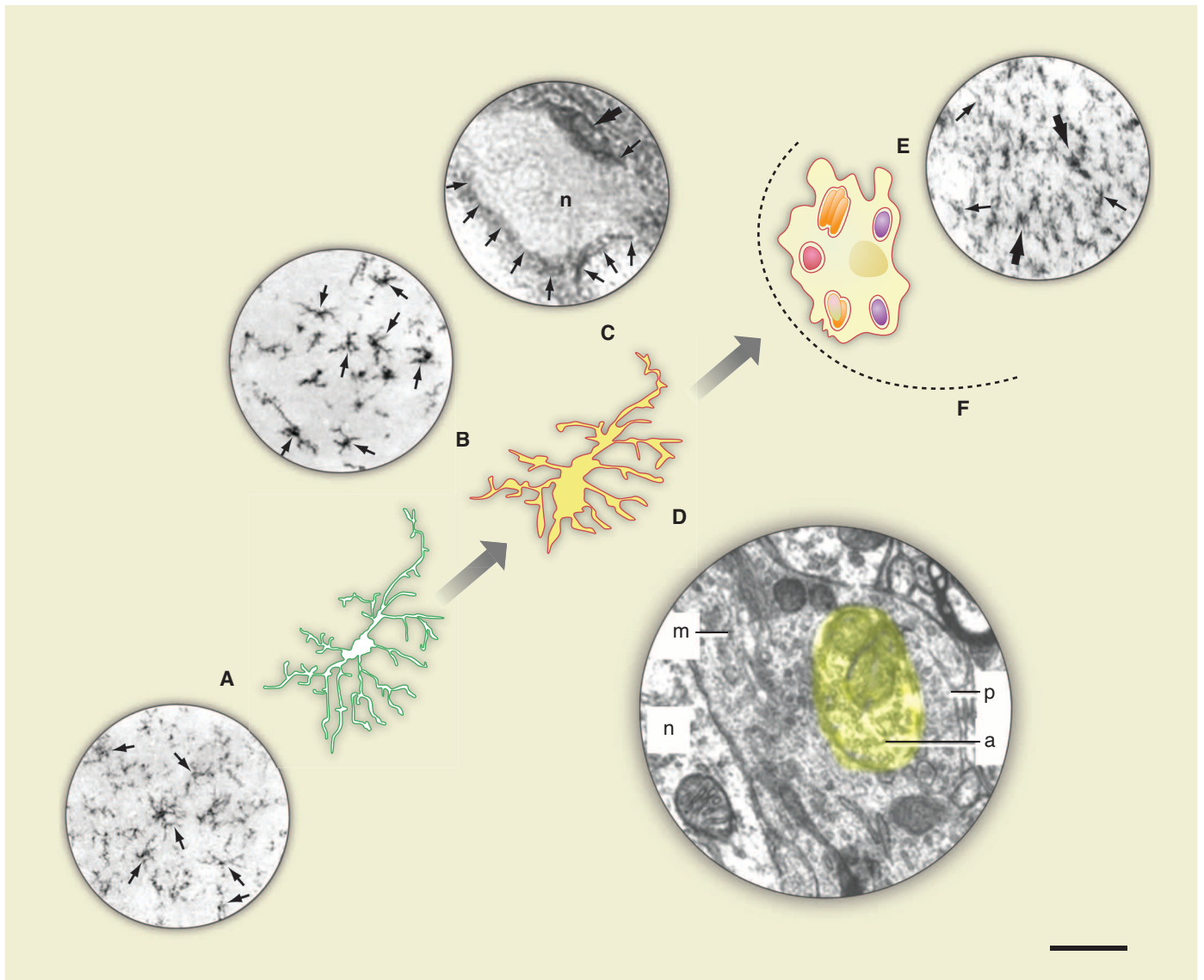


Fig. 1. Schematic drawing showing the microglial activation cascade and associated phenotypic plasticity [modified from (7)]. **(A)** Ramified “resting” microglia (arrows) in the normal rat facial nucleus labeled with the use of the OX-42 monoclonal antibody, which recognizes the rat equivalent of human CD11b, the iC3b complement receptor. **(B)** Activated microglia (arrows), still ramified but with stouter cell processes 24 hours after facial nerve axotomy. **(C)** OX-42 immunoreactive perineuronal microglial cell (large arrow) and microglial processes (small arrows) apposed to a regenerating facial motor neuron (n) 4 days post axotomy [(A to C) taken from figures 1 and 2 of Graeber *et al.* (4)]. **(D)** Image from

day 4 after facial nerve axotomy, a microglial cell process (m) on the surface of a regenerating facial motoneuron (n). Two short pseudopods (p) can be seen arising from the microglial cell process embracing a displaced axonal terminal [a, yellow; from figure 5 of Blinzinger and Kreutzberg (28)]. **(E)** Macrophages can develop from activated microglia, but this transformation is tightly controlled *in vivo*; that is, there is a substantial threshold that needs to be overcome [symbolized in **(F)**]. OX-42 labeling of phagocytic facial nucleus microglia (larger arrows) [taken from figure 2 of Graeber *et al.* (53)]. Scale bar: 50 μm in (A), (B), and (E); 30 μm in (C); 2 μm in (D).

microglia in the trigeminal subnucleus caudalis has been implicated in the central mechanisms of pain associated with dental inflammation (20), and microglial activation has also been observed in the hypothalamic paraventricular nucleus after myocardial infarction (21). There is an intimate association between the spinal microglia-expressed P2X7R and the development of morphine tolerance (22).

The microglial involvement in pain mechanisms may be viewed as an extension of the mi-

croglial sensor function in pathologically altered tissue. Microglia appear to respond to internal and external threats to the entire body that are related but not always limited to the relevant neuronal projection area. Chronic stress increases the number of microglia in certain stress-sensitive brain regions (23). Collectively, microglial cells function as guardians of the brain and spinal cord, acting not only as a tissue alarm system but also exerting defense, as well as repair functions (24).

Strongly activated microglia are capable of producing a broad spectrum of responses to stimuli in the same way as shown for peripheral macrophages (5). However, microglia that react to nociceptive stimuli are not necessarily inflammatory cells. Immune mediators can be produced by microglia under various conditions. Therefore, caution has to be exercised when interpreting this finding as microglial “inflammation.” Increasing evidence suggests that many immune proteins

have brain-specific functions during development and synaptic plasticity (25).

Electricians in the Brain: Microglia Help to Maintain the Integrity of Synapses

In healthy CNS and in the vast majority of known CNS diseases, microglia do not present as macrophages. Therefore, their day-to-day function is different. Interestingly, nonphagocytic activated microglia also show an affinity for neuronal membranes. The initial evidence for this behavior came from studies by Nissl on the relation between nerve cell diseases and glial appearances in the cortex in different psychoses (26). His report on “strung-out, often infinitely long, extremely slim glial cells” that “may cross the entire layer of the large pyramidal neurons” represents the first description of microglia that were discovered in their activated state. Microglial rod cells typically align with apical dendrites, often extending to adjacent neuronal surfaces and even wrapping somatic membranes (Fig. 3). Clinically, cortical rod cells are associated with an acutely dementing process that appears to be reversible, in principle, as demonstrated by the successful treatment of patients suffering from early stages of general paralysis of the insane. Most contemporary researchers will be unfamiliar with microglial rods, because neurosyphilis has become very rare in developed countries. However, general paralysis of the insane affected roughly 1 in 10 hospitalized psychiatric patients in Alois Alzheimer’s time, and he wrote his habilitation thesis on this topic. Other well-known acute psychopathologies associated with microglial rod cells are lead encephalopathy, subacute sclerosing panencephalitis (SSPE), and various forms of viral encephalitis, including HIV-1. The virally induced fusion of microglia to neurons has been demonstrated in an experimental model. The authors argue that the consistent location of the fusion to apical dendrites and the lack of fusion to other neural cell types, both in vitro and in vivo, suggest a unique interaction that may exist between microglia and the dendrites of neurons (27). These dendrites are

normally covered with afferent axon terminals. The displacement of synaptic terminals by microglial cells was first reported in the context of motor neuron regeneration in the brain stem (Fig. 1D) (28), but synaptic stripping also takes place in the cerebral cortex (29), and it probably occurs in the human brain as well (30).

Microglia are not electrotonically coupled like neuroglial cells. This biophysical indepen-

dence probably allows microglia to stay outside the normal activity of neural circuits, enabling them to support the maintenance of synaptic connections analogous to electricians. An electrician maintains and installs electrical equipment but does not form part of the actual circuitry. In line with this idea, Wake *et al.* have proposed that microglia vigilantly monitor and respond to the functional state of synapses (10). This work

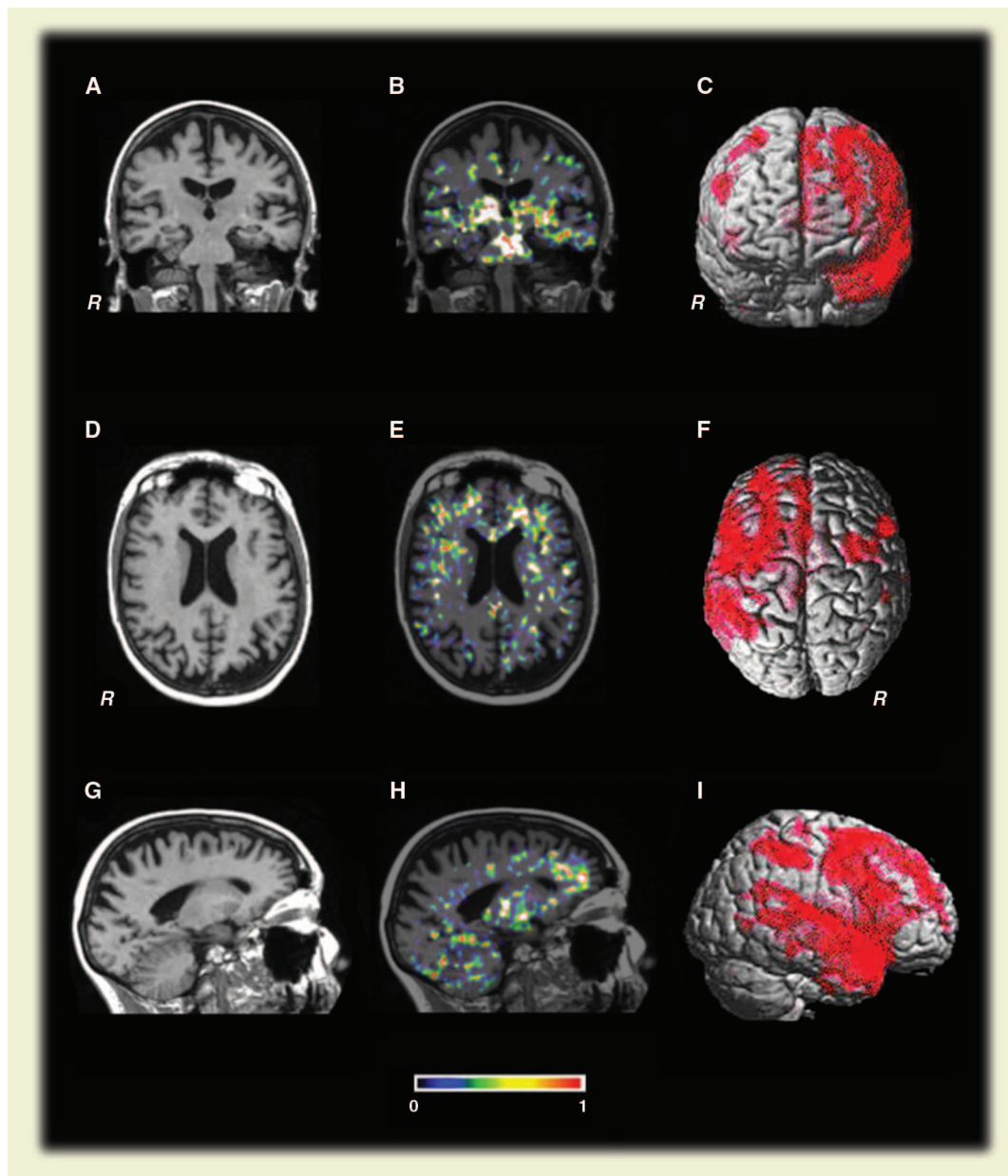


Fig. 2. [^{11}C](R)-PK11195 positron emission tomography images of a patient with predominantly left-hemispheric fronto-temporal lobar dementia (age, 69 years; disease duration, 3 years) (B, E, H) coregistered to the same patient’s magnetic resonance imaging scans (A, D, G). The red in the volume-render magnetic resonance imaging scans (C, F, I) indicates areas of substantial atrophy. These areas overlap with the regional pattern of increased [^{11}C](R)-PK11195 signal. At the bottom of the image, the color bar denotes [^{11}C](R)-PK11195 binding potential values between 0 and 1. The images are shown in radiological convention; that is, the left side of the image is the right of the patient, indicated by R. (F) Top-down view onto the cortex with the right side of the image also being the right of the patient. (G and H) Sagittal sections through the left hemisphere. [Courtesy of R. B. Banati, Sydney (54)]

demonstrates that microglia sense defunct synapses and eliminate them, a finding that is highly reminiscent of the posttraumatic synaptic stripping that affects motoneurons, as reported by Blinzinger and Kreutzberg more than 40 years ago (28). Interestingly, the classical complement cascade has been suggested to mediate the elimination of synapses in the CNS (31), and microglia involved in synaptic stripping strongly up-regulate complement receptors (4). However, there is no visible phagocytosis of axon terminals by microglia in either model (10, 28), rendering retraction of the unused axon terminals a possibility. All resting microglia express complement receptor 3 (CD11b) (21), but additional pathways are likely to be involved in microglia-synaptic interactions. Microglial cells may also control synaptogenesis. This is suggested by the observation that a mutation in KARAP/DAP12, a key protein of microglial activation, influences synaptic functions in the hippocampus, as well as synaptic protein content (32). Moreover, it has been demonstrated that microglial brain-derived neurotrophic factor directly regulates synaptic properties in the spinal cord (18, 32).

Diseases That Make Microglia Sick

Neurodegenerative diseases are common and are caused by primary dysfunction and subsequent death of nerve cells. There are compelling reasons to complement this concept with that of primary gliodegeneration. This terminology is clearly preferable over “non-cell-autonomous degeneration,” because the latter needs to be distinguished from glial non-cell-autonomous effects on neurons (that is, glial gain-of-function mutations that are toxic on nerve cells). Microglial non-cell-autonomous effects on neurons have been reported in a number of conditions. Microglial gain-of-function mutations: Mutant mSOD1G93A (where G93A is Gly⁹³ → Ala⁹³) microglia are cytotoxic (33), a phenomenon long known from virus-infected mononuclear phagocytes that secrete neurotoxins when infected with HIV-1. Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate (34). However, microglial loss of function (“insufficiency”) also occurs. An example of microglial loss-of-function mutations can be found in Nasu-Hakola disease, which is a cognitive disorder caused by a primary defect of CNS microglia (35). It is due to a mutation in the *DAP12* or *TREM2* gene (36). The adaptor protein DAP12 is critical for the activity of mononuclear phagocytes. DAP12-mutant mice and humans show

dysfunction of osteoclasts and microglia, as well as bone and brain abnormalities (37). Otero *et al.* (37) found substantially fewer microglia in the basal ganglia and the spinal cord of older DAP12-deficient mice, and those detectable showed extensive cytoplasmic fragmentation (cytorrhesis) and nuclear condensation characteristic of microglial degeneration and apoptotic cell death. Microglia are also affected in Creutzfeldt-Jakob disease (Fig. 4A), a noninfectious but transmissible condition, which may explain the failure to detect microglial involvement in the synaptic loss observed in prion disease (38). Thus, primary as well as secondary microgliopathic states have to be considered an alternative to “microglial activation” when phenotypic changes of microglia in pathological tissue are analyzed.

Is the Only Bad Microglial Cell a Dead Microglial Cell?

Microglia can kill exogenous pathogens. They are the cellular defense system of the brain and the spinal cord. Microglia also play a role in developmental neuronal death: for example, in the hippocampus, which requires the microglial CD11b integrin and the DAP12 immunoreceptor (39). Diseased microglia can turn into aggressors, as

mentioned earlier, but the fact that microglial insufficiency itself causes disease provides a strong argument in favor of normal microglia being necessary and exerting a neurosupportive, rather than a neurotoxic, effect by default. Lack of preservation of tissue structure is an important determinant for the formation of microglia-derived macrophages. If there is extensive cellular damage or tissue necrosis, only phagocytic microglia/macrophages can be found. However, as the example of the microglial rod cell shows, the interaction of activated microglia with nerve cells does not automatically result in killing and phagocytosis. This sparing of neurons by reactive microglia also argues against the view that microglial cells are a threat to nerve cells as soon as they become activated. Indeed, synaptic stripping is associated with regeneration of motor neurons (28) and probably serves a protective function in the inflamed cerebral cortex (29). Therefore, the catchphrase statement that the only bad microglial cell is a dead microglial cell (24) appears justified in principle. For instance, in Alzheimer’s disease, dystrophic (senescent), rather than activated, microglial cells are associated with tau pathology and likely precede neurodegeneration (Fig. 4C) (40). The emphasis in the literature on microglial neurotoxic prop-

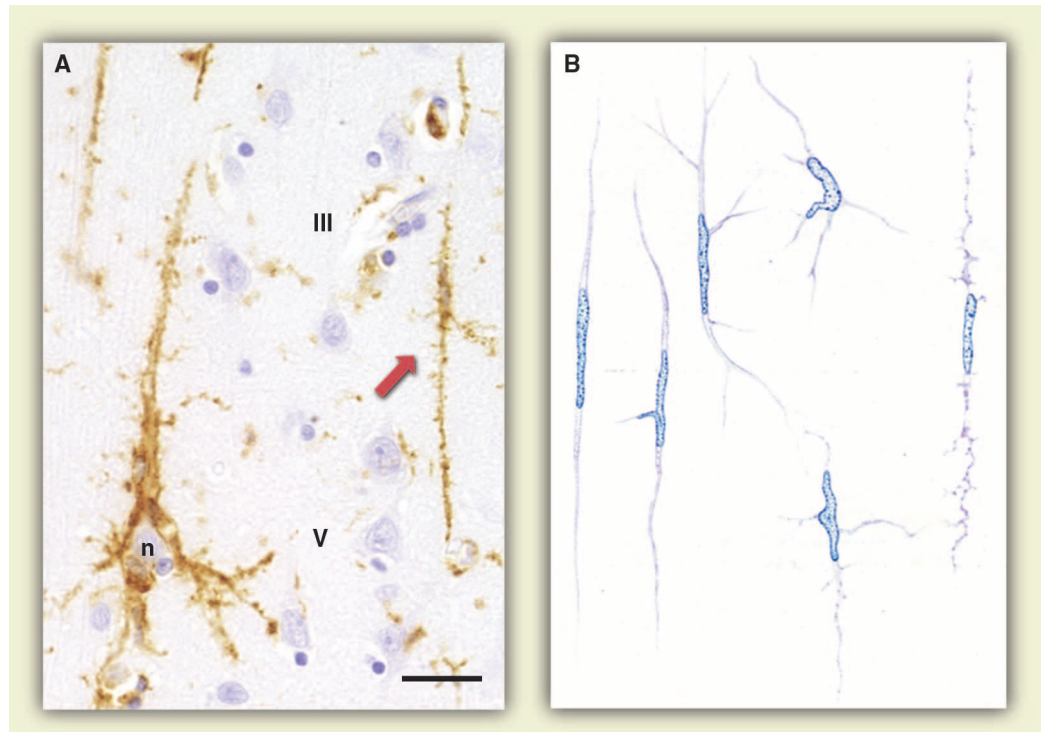


Fig. 3. Microglial rod cells expressing MHC class II molecules in a case of SSPE (A) and rod cells (B), as depicted by Spielmeyer (55). The arrow in (A) points to a single rod cell. “n” denotes a pyramidal neuron that is tightly wrapped by microglial cell processes; “III” and “V” indicate cortical layers. Human cerebral cortex is labeled with the use of the CR3/43 monoclonal antibody that recognizes the beta-chain of HLA-DR, DQ, and DP. Microglial rod cells arguably represent the most intriguing microglial phenotype, because they are typically found in the cerebral cortex in association with cognitive symptoms; they show a great affinity for neuronal surfaces and, notably, dendrites; they are not normally phagocytic; they adjust their shape to an extreme extent; and they represent the longest known but least investigated microglial phenotype. Scale bar: 60 μ m.

erties seems arbitrary and more related to the high prevalence of Alzheimer's and Parkinson's diseases than actual pathophysiological evidence confirming that microglia pose a threat to neurons *in vivo*. It only adds to the confusion surrounding microglial neurotoxicity that the claim is often made in conjunction with the proposal that the expression of molecules that are of immunological importance in peripheral organs constitutes evidence of microglial "inflammation," in the absence of cells of the peripheral immune system. Current data support the idea that inflammatory mediators, which enhance the phenotypic and immunological activation of glia, do not promote A β accumulation but limit A β deposition (41). Therefore, we agree that the loss of microglial normal functions is sufficient to explain a range of disease conditions without the need to interpret microglial expression of immune molecules as evidence of inflammation.

Abnormal Behavior Caused by Mutant Microglia

Hoxb8 mutant mice show behavior reminiscent of humans with the obsessive-compulsive disorder trichotillomania (that is, these mice compulsively remove their hair). In these animals, the behavioral disorder is associated with mutant microglia (42). However, only a subpopulation of microglia that originate postnatally in the bone marrow and predominantly populate brain regions relevant to the syntactic groom chain (such as the cortex, striatum, and brainstem) are affected. This anatomical selectivity seems surprising at first but is not unheard of in relation to microglia: Although osteoclasts and spleen macrophages are entirely dependent on macrophage colony-stimulating factor (M-CSF), microglia require M-CSF only in specific areas of the CNS, and DAP12 deficiency exclusively affects microglia in restricted locations in the CNS, such as the basal ganglia and spinal cord (37). It is also worth noting that microglia display brain region-specific functional diversity (43). The Hoxb8 study is of major importance, because it directly links functional insufficiency of bone marrow-derived microglia to abnormal behavior.

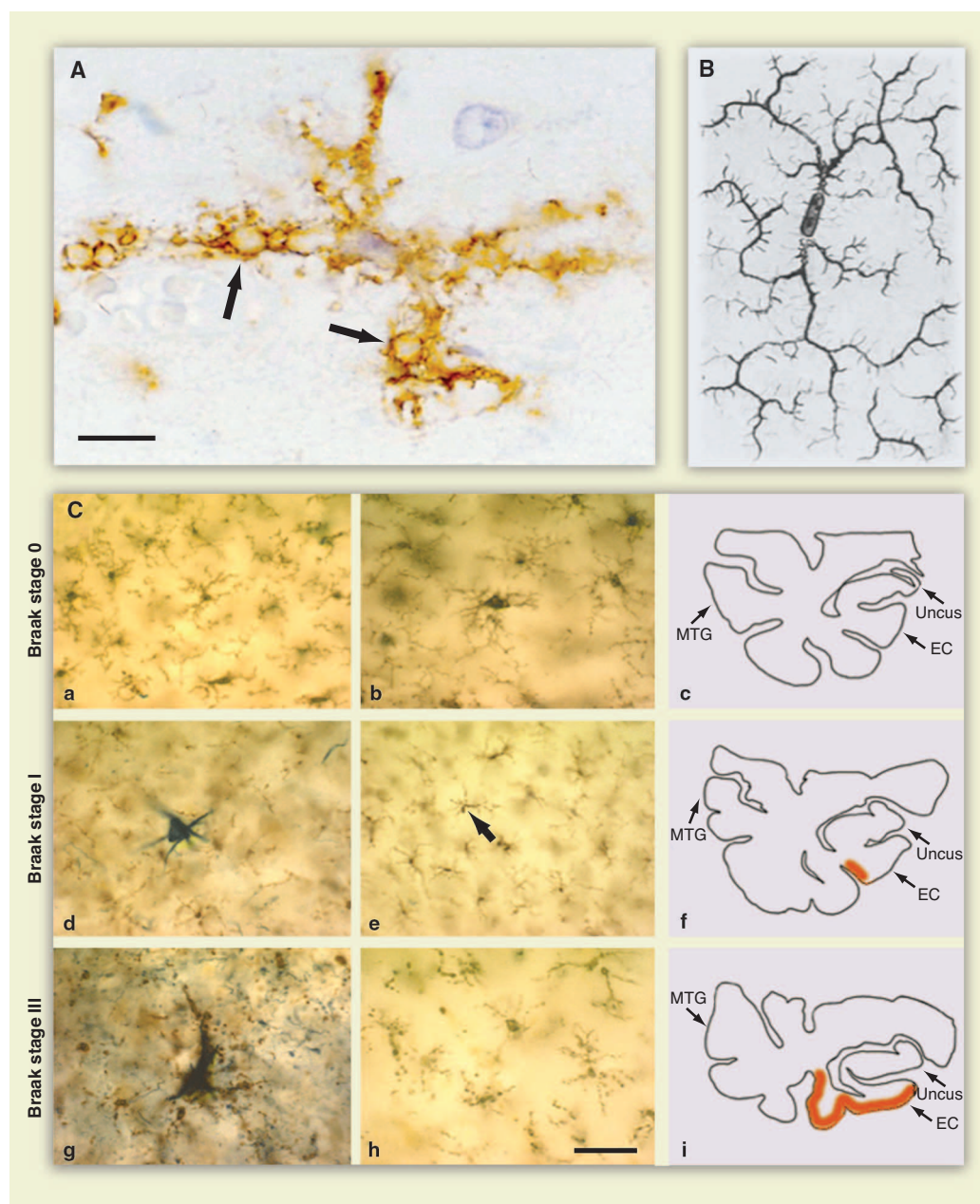


Fig. 4. (A) MHC class II immunoreactive "sick" microglial cell severely affected by spongiform change in Creutzfeldt-Jakob disease. The arrows point to large vacuoles that have destroyed the normal structure of the microglial cell processes. Scale bar: 10 μ m. [Reproduced with permission from the *Journal of Neuropathology and Experimental Neurology* (56)] (B) Rio-Hortega's drawing of a microglial cell (57). There are no vacuoles in normal state [compare with (A)], nor is there any fragmentation of cell processes [compare with (C)]. (C) Microglial fragmentation precedes the spread of tau pathology in the temporal lobe of Alzheimer's patients. Double-label immunohistochemistry for microglia (iba1) and tau (AT8) is shown in three subjects with tau pathology increasing from Braak stage 0 to stage III. Camera lucida drawings of the actual sections are shown in (c), (f), and (i), indicating the uncus for orientation purposes, as well as both sampling areas in the entorhinal cortex (EC) and the middle temporal gyrus (MTG); areas of tau pathology are shaded orange. Representative micrographs of the EC (a, d, g) and MTG (b, e, h) reveal microglia (brown) and tau pathology (black) at the different stages. Normal ramified microglia are evident at stage 0 in both EC and MTG in the absence of tau pathology (a, b). Mostly fragmented microglia are seen in association with a neurofibrillary tangle and neuropil threads in (d), whereas mostly ramified and only a single fragmented cell (arrow) are present in (e) during stage I. Severe microglial fragmentation and loss of discernable cell shape are colocalized with extensive tau pathology in (g); microglial processes are fragmented also in (h) in the absence of neurodegeneration, but cells retain recognizable contours. Scale bar: 50 μ m (a, b, d, e, g, h). [Reproduced from (40) with permission]

Microglia may be involved in other psychiatric conditions. For instance, there is evidence for activation of microglia in the brains of patients with schizophrenia and affective disorders (44). It has been suggested that schizophrenia is a disease that develops because of derangements to human-specific CNS functions that have emerged since our species diverged from non-human primates (45). The sialic acid-recognizing immunoglobulin-superfamily lectin SIGLEC11—which is expressed in human, but not in chimpanzee, brain microglia (13)—was mentioned above. The neural cell adhesion molecule (NCAM) is the predominant carrier of α 2,8-polysialic acid (PSA) in the mammalian brain, and abnormalities in PSA and NCAM expression are associated with schizophrenia in humans and cause deficits in hippocampal synaptic plasticity and contextual fear conditioning in mice (46).

A Future for Self-Donated Bone Marrow-Derived Microglia

Although there are still questions today in regard to the number of microglial subpopulations in the CNS, it was demonstrated conclusively about a decade ago that ramified CNS microglia can derive from bone marrow precursors, even in adults (47). This finding immediately suggested possible new therapeutic avenues, and there are now reports on the successful treatment of CNS diseases by means of bone marrow transplantation. For instance, lentivirus-mediated gene therapy of hematopoietic stem cells has been used with great success in two 7-year-old boys suffering from a rare and fatal brain demyelinating disease (48). The ex vivo genetically modified cells were able to reach the CNS and stopped the progressive cerebral demyelination in the two patients, demonstrating the power of such cellular therapy for brain diseases. The Hoxb8 mutant microglia study (42) demonstrates that transplantation of normal bone marrow can efficiently rescue the Hoxb8 mutant grooming phenotypes, including restoration of hairless patches, healing of open lesions, and reduction of excessive grooming times back to normal. Bone marrow-derived cells further contribute to the recruitment of microglial cells in response to β -amyloid deposition in APP/PS1 double transgenic Alzheimer's mice (49), and it has been suggested that bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease (50). Infiltration of bone marrow-derived microglia in high-grade gliomas (2, 12) is also of potential therapeutic importance. Consequently, autologous bone marrow-derived microglia have developed into

an attractive target for genetic modification although there are a number of technical issues that still need to be addressed (51).

Conclusions

Microglia have come a long way within 20 years, from their existence being questioned (1) to providing a potentially very elegant handle on the treatment of higher brain-functional defects (42). Yet there are still many white spots on the microglia map: One is the precise extent of microglial turnover in humans (52). Trafficking of microglia precursors across the blood-brain barrier could cause brain dysfunction associated with systemic infection. When we feel unwell during a bout of fever, are microglial cell processes becoming wobbly in the brain, weakening their potentially stabilizing effect on neural circuits? Clearly, microglial involvement in synaptic plasticity should become a focus of research. Whether bone marrow-derived microglia (for example, in the brain of Hoxb8-animals) fuse with neurons is unknown, but fusion of macrophages is not a rare event. Glial cells are now accepted to provide more to neurons than mere structural and nutritional support, but genetically modified bone marrow-derived microglia precursors could provide a unique route of entry into the CNS. Genetically enhanced (that is, reprogrammed) microglia might revolutionize the treatment of CNS diseases, one step short of synthetic biology. Thus, the current focus on microglial inflammation as a cause of neurodegeneration appears misplaced.

References and Notes

- C. L. Dolman, in *Textbook of Neuropathology*, R. L. Davis, D. M. Robertson, Eds. (Williams & Wilkins, Baltimore, MD, 1991), pp. 141–163.
- A. Sica et al., *Semin. Cancer Biol.* **18**, 349 (2008).
- G. W. Kreutzberg, *Trends Neurosci.* **19**, 312 (1996).
- M. B. Graeber, W. J. Streit, G. W. Kreutzberg, *J. Neurosci. Res.* **21**, 18 (1988).
- C. A. Colton, D. M. Wilcock, *CNS Neurol. Disord. Drug Targets* **9**, 174 (2010).
- T. Morioka, A. N. Kalehua, W. J. Streit, *J. Cereb. Blood Flow Metab.* **11**, 966 (1991).
- M. B. Graeber, in *Encyclopedia of Neuroscience*, G. Adelman, B. H. Smith, Eds. (Elsevier, Amsterdam, ed. 3, 2004), article 335.
- E. A. Eugenin et al., *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4190 (2001).
- G. J. Liu, R. Nagarajah, R. B. Banati, M. R. Bennett, *Eur. J. Neurosci.* **29**, 1108 (2009).
- H. Wake, A. J. Moorhouse, S. Jinno, S. Kohsaka, J. Nabekura, *J. Neurosci.* **29**, 3974 (2009).
- K. Williams, X. Alvarez, A. A. Lackner, *Glia* **36**, 156 (2001).
- M. B. Graeber, W. J. Streit, *Acta Neuropathol.* **119**, 89 (2010).
- T. Hayakawa et al., *Science* **309**, 1693 (2005).
- A. Salminen, K. Kaarmiranta, *J. Mol. Med.* **87**, 697 (2009).
- A. Varki, *Proc. Natl. Acad. Sci. U.S.A.* **107** (suppl. 2), 8939 (2010).

- A. W. Lemstra et al., *J. Neuroinflammation* **4**, 4 (2007).
- H. S. Smith, *Pain Physician* **13**, 295 (2010).
- J. A. Coull et al., *Nature* **438**, 1017 (2005).
- Y. X. Chu, Y. Zhang, Y. Q. Zhang, Z. Q. Zhao, *Brain Behav. Immun.* **24**, 1176 (2010).
- W. Fan et al., *Arch. Oral Biol.* **55**, 706 (2010).
- I. Rana et al., *Brain Res.* **1326**, 96 (2010).
- D. Zhou, M. L. Chen, Y. Q. Zhang, Z. Q. Zhao, *J. Neurosci.* **30**, 8042 (2010).
- R. J. Tynan et al., *Brain Behav. Immun.* **24**, 1058 (2010).
- W. J. Streit, Q. S. Xue, *J. Neuroimmune Pharmacol.* **4**, 371 (2009).
- L. M. Boulanger, *Neuron* **64**, 93 (2009).
- F. Nissl, *Archiv für Psychiatrie* **32**, 656 (1899).
- J. B. Ackman, F. Siddiqi, R. S. Walikonis, J. J. LoTurco, *J. Neurosci.* **26**, 11413 (2006).
- K. Blinzinger, G. Kreutzberg, *Z. Zellforsch. Mikrosk. Anat.* **85**, 145 (1968).
- B. D. Trapp et al., *Glia* **55**, 360 (2007).
- M. B. Graeber, K. Bise, P. Mehraein, *Acta Neuropathol.* **86**, 179 (1993).
- B. Stevens et al., *Cell* **131**, 1164 (2007).
- A. Bessis, C. Béchade, D. Bernard, A. Roumier, *Glia* **55**, 233 (2007).
- D. R. Beers et al., *Proc. Natl. Acad. Sci. U.S.A.* **103**, 16021 (2006).
- I. Maezawa, L. W. Jin, *J. Neurosci.* **30**, 5346 (2010).
- J. C. Thrash, B. E. Torbett, M. J. Carson, *Neurochem. Res.* **34**, 38 (2009).
- M. Kaneko, K. Sano, J. Nakayama, N. Amano, *Neuropathology* **30**, 463 (2010).
- K. Otero et al., *Nat. Immunol.* **10**, 734 (2009).
- Z. Šišková, A. Page, V. O'Connor, V. H. Perry, *Am. J. Pathol.* **175**, 1610 (2009).
- S. Wakselman et al., *J. Neurosci.* **28**, 8138 (2008).
- W. J. Streit, H. Braak, Q. S. Xue, I. Bedchmann, *Acta Neuropathol.* **118**, 475 (2009).
- P. Chakrabarty et al., *FASEB J.* **24**, 548 (2010).
- S. K. Chen et al., *Cell* **141**, 775 (2010).
- A. H. de Haas, H. W. Boddeke, K. Biber, *Glia* **56**, 888 (2008).
- T. A. Bayer, R. Buslei, L. Havas, P. Falkai, *Neurosci. Lett.* **271**, 126 (1999).
- B. Dean, *Aust. N. Z. J. Psychiatr.* **43**, 13 (2009).
- G. Kochlamazashvili et al., *J. Neurosci.* **30**, 4171 (2010).
- A. Flügel, M. Bradl, G. W. Kreutzberg, M. B. Graeber, *J. Neurosci. Res.* **66**, 74 (2001).
- N. Cartier et al., *Science* **326**, 818 (2009).
- T. Malm et al., *Neurobiol. Dis.* **18**, 134 (2005).
- A. R. Simard, D. Soulet, G. Gowing, J. P. Julien, S. Rivest, *Neuron* **49**, 489 (2006).
- D. Soulet, S. Rivest, *Curr. Opin. Pharmacol.* **8**, 508 (2008).
- E. R. Unger et al., *J. Neuropathol. Exp. Neurol.* **52**, 460 (1993).
- M. B. Graeber, W. J. Streit, R. Kiefer, S. W. Schoen, G. W. Kreutzberg, *J. Neuroimmunol.* **27**, 121 (1990).
- A. Cagnin, M. Rossor, E. L. Sampson, T. MacKinnon, R. B. Banati, *Ann. Neurol.* **56**, 894 (2004).
- W. Spielmeier, *Histopathologie des Nervensystems* (Springer, Berlin, 1922).
- U. V. Eitzen et al., *J. Neuropathol. Exp. Neurol.* **57**, 246 (1998).
- P. del Rio-Hortega, *Bol. de la Soc. Esp. de Biol.* **9**, 68 (1919).
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Electron-Like Scattering of Positronium

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According to our current understanding of nature, every charged particle has an antimatter counterpart of the same mass but opposite charge with which it may annihilate. The antimatter counterpart to the electron is the positron. Its interaction with matter is used to shed light onto fundamental physics, probe material defects, and perform dynamic imaging of metabolic pathways through positron emission tomography (PET).

In many positron collisions with matter, positronium (Ps), a hydrogen-like atom of an electron and positron, is formed. For example, over 80% of all annihilation γ -rays produced in PET scans (1), and 90% of those emanating from the center of our galaxy (2), arise from Ps decay. Its lifetime is millions of times longer than typical atomic scattering times, so Ps has ample time to interact with matter once formed; however, information on its scattering properties remains scarce, and full theoretical descriptions are beyond reach for all but the simplest targets (i.e., H and He).

We have found that the Ps total cross section (a measure of its overall interaction probability) is unexpectedly close to that of a bare electron moving at the same velocity, despite Ps being neutral and having twice the mass (Fig. 1 and fig. S1).

Before this work, Ps had been expected to scatter rather differently from the electron. The coincidence of the centers of mass and charge in Ps has been expected to give rise to a negligible static interaction (that with an undistorted target),

and its neutrality thought to result in a much weaker and shorter ranged van der Waals force (the attraction to another neutral polarizable system such as an atom or molecule). Hence, the short-range distortions arising from electron exchange between Ps and the target have been thought to be of greater consequence than for electrons [e.g., (3)].

Total cross sections for the electron and Ps are plotted in Fig. 1, and the similarity is evident throughout the velocity range investigated (0.5 to 2.0 a.u. where 1 a.u. ~ 2190 km s $^{-1}$) and for a wide variety of atomic and molecular targets (He, Ne, Ar, Kr, Xe, H $_2$, N $_2$, O $_2$, H $_2$ O, and SF $_6$). No drastic deviations of the Ps cross section from its electron counterpart have been observed, even when approaching velocities where phenomena very sensitive to the details of the interaction occur in the case of electrons (namely, shape resonances and Ramsauer-Townsend minima). Shape resonances, not yet observed for positrons but exemplified by the peaks in the electron data below 1.0 a.u. for N $_2$ and SF $_6$ (Fig. 1), arise from the temporary attachment of the projectile to the target. Ramsauer-Townsend minima take place when the scattered electron wave function is pulled sufficiently by the target as to make it “in step” with the incident wave so that, at certain velocities, the target appears transparent (e.g., the minimum in the electron measurements for Kr in Fig. 1). Whether these phenomena occur for a Ps impact remains to be established.

Also shown in Fig. 1 are available theories for Ps scattering. Calculations for He, performed via

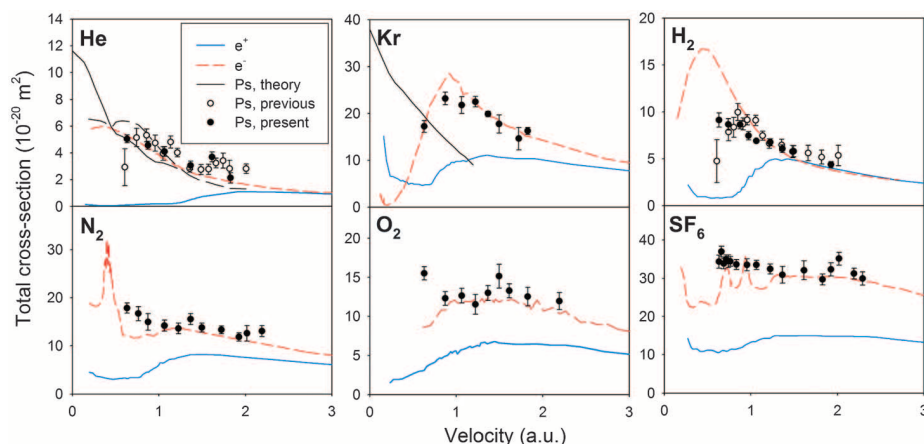


Fig. 1. Total cross sections for various targets, illustrating the electron-like behavior of Ps. The present (●) and previous (○) (7) experimental results for Ps are compared with experimental positron (blue) (8) and electron data (red) (8) and available Ps theory (black lines): He, solid and dashed lines (3, 9); Kr, solid line (10). The legend applies to all graphs. Error bars indicate SEM.

close-coupling methods, are in fair agreement with experiment at intermediate velocities. As to the source of this electron-like behavior, some clues may come from theoretical investigations of Ps breakup on He. A Monte Carlo calculation (4) suggested that the polarization of the Ps by the screened Coulomb field of the nucleus may result in the electron being closer on average than the positron. An impulse approximation (5), which agrees well with experiment, used a coherent sum of the separate scattering amplitudes of the positron and electron in the Ps. The present results would then suggest that the positron scattering amplitude is significantly smaller than that of the electron. Further experimental and theoretical activities are eagerly anticipated.

In the meantime, we propose that recourse be made to the extensive database available for electron scattering for initial estimates of the magnitude of Ps total cross sections in, for example, positron-propagation calculations, whether in the interstellar medium (2) or for accurate dosimetry in PET (1). Given the role of low-energy resonances by secondary electrons to radiation damage in biological tissue (6), the ramifications of the present results might be far-reaching.

References and Notes

1. C. Champion, C. Le Loirec, *Phys. Med. Biol.* **52**, 6605 (2007).
2. R. L. Kinzer et al., *Astron. Astrophys. Suppl. Ser.* **120**, 317 (1996).
3. J. E. Blackwood, C. P. Campbell, M. T. McAlinden, H. R. J. Walters, *Phys. Rev. A* **60**, 4454 (1999).
4. L. Sarkadi, *Phys. Rev. A* **68**, 032706 (2003).
5. C. Starrett, M. McAlinden, H. R. J. Walters, *Phys. Rev. A* **72**, 012508 (2005).
6. B. Boudaïffa, P. Cloutier, D. Hunting, M. A. Huels, L. Sanche, *Science* **287**, 1658 (2000).
7. A. J. Garner, G. Laricchia, A. Özen, *J. Phys. B* **29**, 5961 (1996).
8. W. E. Kauppila, T. S. Stein, in *Advances in Atomic, Molecular and Optical Physics*, D. Bates, B. Bederson, Eds. (Academic Press, San Diego, CA, 1990), vol. 26, pp. 1–50.
9. A. Basu, P. K. Sinha, A. S. Ghosh, *Phys. Rev. A* **63**, 052503 (2001).
10. J. E. Blackwood, M. T. McAlinden, H. R. J. Walters, *J. Phys. B* **35**, 2661 (2002).
11. We thank R. Drachman, M. Shipman, J. Tennyson, and J. Walters for useful discussions and J. Dumper and R. Jawad for their technical assistance. Engineering and Physical Sciences Research Council (UK) is thanked for supporting this work.

Supporting Online Material

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Materials and Methods

Fig. S1

References

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Channel-Mediated Tonic GABA Release from Glia

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Synaptic inhibition is based on both tonic and phasic release of the inhibitory transmitter γ -aminobutyric acid (GABA). Although phasic GABA release arises from Ca^{2+} -dependent exocytosis from neurons, the mechanism of tonic GABA release is unclear. Here we report that tonic inhibition in the cerebellum is due to GABA being released from glial cells by permeation through the Bestrophin 1 (Best1) anion channel. We demonstrate that GABA directly permeates through Best1 to yield GABA release and that tonic inhibition is eliminated by silencing of Best1. Glial cells express both GABA and Best1, and selective expression of Best1 in glial cells, after preventing general expression of Best1, fully rescues tonic inhibition. Our results identify a molecular mechanism for tonic inhibition and establish a role for interactions between glia and neurons in mediating tonic inhibition.

A tonic form of synaptic inhibition that causes sustained activation of γ -aminobutyric acid (GABA) receptors in neurons (1) occurs throughout the central nervous system (2–4). Because of its sustained nature, tonic inhibition dominates over conventional (phasic) synaptic inhibition in controlling neuronal excitability (1). Therefore, tonic inhibition plays an important role in neuronal information processing (5) and has been implicated in epilepsy, sleep, memory, and cognition (6–8). The mechanism underlying the tonic release of GABA and the source of this GABA are poorly understood. We have addressed this question in cerebellar granule cells, which are powerfully restrained by tonic inhibition resulting from GABA released via an unconventional mechanism that is independent of action potentials and does not require vesicular exocytosis (9, 10).

We hypothesized that tonic inhibition is mediated by GABA permeating through an anion channel, Best1 (11), previously implicated in glutamate release from astrocytes (12). Best1 has several features that make it attractive as a hypothetical mediator of tonic GABA release. Best1 is an anion channel that is activated by intracellular Ca^{2+} and by changes in cell volume, though it is tonically active even at resting Ca^{2+} levels and at normal cell volume. Best1 has unique permeability properties among anion channels, with a significant permeability to HCO_3^- ($P_{\text{HCO}_3^-}/P_{\text{Cl}^-} = 0.44$) (13) and a much higher permeability to large anions,

such as SCN^- , than to Cl^- (14). This channel is even permeable to gluconate ($P_{\text{gluconate}^-}/P_{\text{Cl}^-} = 0.4$) (14) and to the neurotransmitter, glutamate (12). Thus, Best1 might be permeable to GABA and thereby mediate tonic GABA release.

Best1 is a GABA-permeable channel. We first determined the permeability of Best1 to GABA (15). Best1 channels were expressed in human embryonic kidney–293 (HEK293T) cells, and whole-cell patch-clamp measurements were used to determine the reversal potential of the resulting ionic currents. Best1 channels were maximally activated by high ($\sim 4.5 \mu\text{M}$) free Ca^{2+} solution dialyzed into the cell upon establishment of the whole-cell configuration. The voltage dependence of currents flowing through Best1 was determined as illustrated in Fig. 1A (black trace). The reversal potential of the Best1-mediated current was $+2.9 \pm 4.4 \text{ mV}$ ($n = 5$), which closely matched the equilibrium potential of $+1 \text{ mV}$ predicted for Cl^- . This indicates a current carried by Cl^- under these conditions. This current was not observed in the presence of an anion channel blocker, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), and was very small in the complete absence of internal Ca^{2+} (Fig. 1, A and B). Two lines of evidence indicate that this current is caused by Best1. First, currents were absent in cells that were not transfected with Best1 (fig. S1). Second, making a tryptophan-to-cysteine residue mutation (W93C) in Best1 that is known to block the pore of the Best2 anion channel (16) eliminated all Ca^{2+} -activated current (Fig. 1, A and B). Thus, Best1 behaves as an anion channel, consistent with previous conclusions (17).

The GABA permeability of the Best1 channel was examined by replacing Cl^- in the intracellular solution with GABA (Fig. 1C). The reversal potential of the current varied with intracellular GABA concentration (Fig. 1D). These shifts in reversal potential indicate that the GABA permeability of Best1 is substantial, although less than that of

Cl^- . At an intracellular GABA concentration of 120 mM, the permeability of GABA relative to Cl^- ($P_{\text{GABA}}/P_{\text{Cl}^-}$) was 0.27. Even though GABA is predominantly zwitterionic, the amount of GABA in anionic form is sufficient to carry considerable current, as indicated by current flow when GABA is the only permeant species (fig. S1).

Next, the Ca^{2+} dependence of the GABA flux was examined by measuring current (at -80 mV) produced by various intracellular Ca^{2+} concentrations (Fig. 1E). The half-maximal Ca^{2+} concentration was calculated to be 150 nM with a Hill coefficient of 1.4 (Fig. 1F). Thus, GABA efflux is substantial even at basal cytosolic Ca^{2+} levels of about 100 nM (18). The reversal potential of this current was identical at all intracellular Ca^{2+} concentrations, which indicated that GABA permeability is unaffected by Ca^{2+} (18).

To determine whether such GABA efflux through Best1 can be detected by a neighboring cell, we developed a two-cell biosensor micro-assay. GABA released through Best1 channels expressed in one HEK293T cell was detected via a second HEK293T cell that expressed GABA subtype C (GABA_C) receptors (Fig. 2A). Unlike the heteromeric GABA_A receptors that mediate tonic inhibition in hippocampus and cerebellum (19), the GABA_C receptor that mediates tonic inhibition in the retina (20) forms homomeric channels after expression of only a single subunit (21). GABA_C receptors have a high affinity for GABA (22) and slow desensitization kinetics (23), which facilitate detection of tonic GABA release (20). The internal solution for cells expressing Best1 contained 3 or 140 mM GABA, and Best1 was activated by Ca^{2+} concentrations of 100 nM or 4.5 μM .

We confirmed that Best1 displayed a significant permeability to GABA (Fig. 2B, top trace), indicated by an inward current flow at -80 mV , and that this current was associated with release of GABA onto the sensor cell (Fig. 2B, bottom trace). GABA release depended on permeation through Best1, because it was absent in conditions that block Best1, such as NPPB treatment and the W93C mutation (Fig. 2, C and D). To quantify the amount of GABA release, we normalized the response to the maximal current produced by bath application of 100 μM GABA (Fig. 2B, inset). With this approach we could compare GABA release under a variety of conditions (Fig. 2E) and reach a number of conclusions. First, GABA release was very small when Best1 was not expressed and could not be detected from cells expressing the W93C mutation. Second, GABA release was evident even at resting intracellular Ca^{2+} concentration (100 nM) and could be detected when intracellular GABA concentration was as low as 3 mM. At high intracellular concentrations of Ca^{2+} (4.5 μM) and GABA (140 mM), the amount of GABA released was so large that it nearly saturated (80% maximal) GABA_C receptors on the sensor cells. Third, GABA release was completely abolished by the anion channel blockers NPPB and niflumic acid (NFA); this was not due to a direct action of these com-

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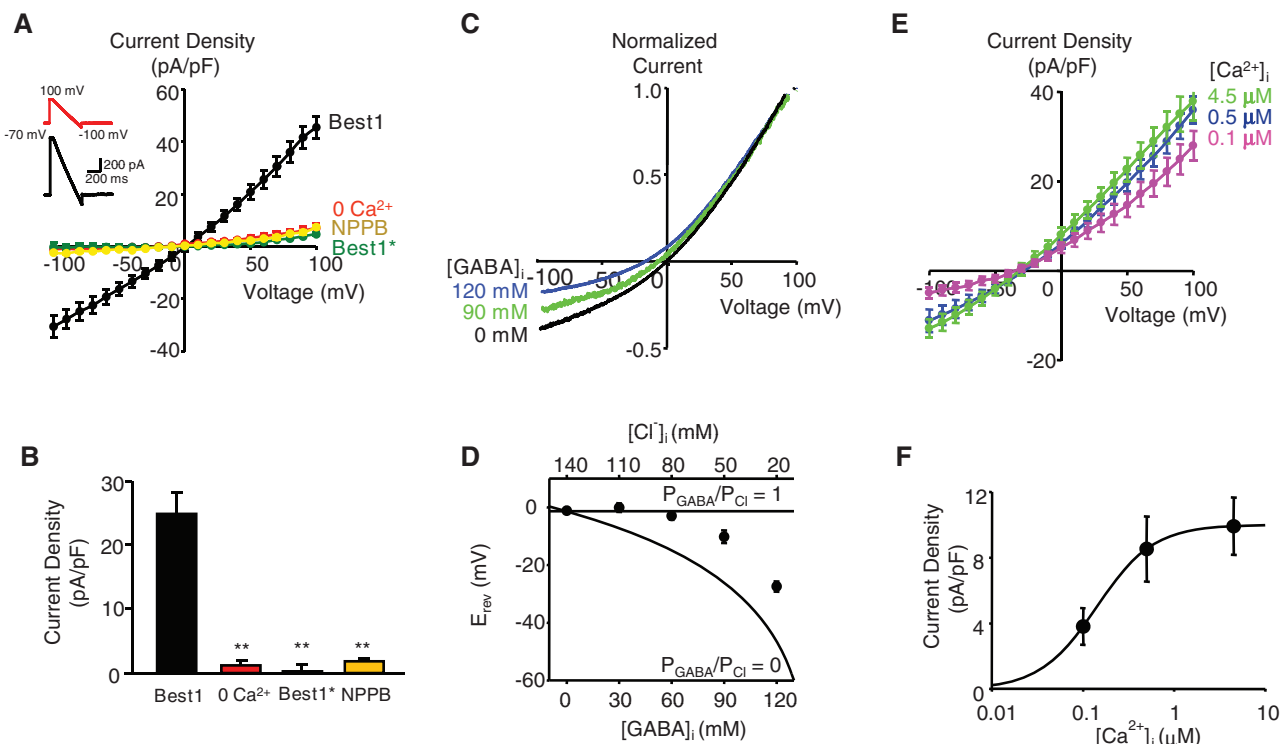


Fig. 1. Best1 is a GABA-permeable anion channel. **(A)** Relations between membrane potential (voltage) and mean current density measured in HEK293T cells expressing Best1 in the absence (0 Ca²⁺, $n = 5$) and presence of Ca²⁺ (~4.5 μM, $n = 5$). Current density was greatly reduced by expressing Best1 with a channel pore mutation, Best1-W93C (Best1*, $n = 5$), or by treatment with the anion channel blocker NPPB (100 μM, $n = 6$). Inset shows the protocol used to measure the relations between current (black) and voltage (red); in all cases intracellular Cl⁻ concentration was 154 mM. **(B)** Summary of mean current density measured at -80 mV for each condition in (A). Throughout this research article, numerical values indicate means ± SEM unless otherwise indicated. Asterisks above bars indicate a significant difference determined by unpaired two-tailed t test ($P < 0.01$). **(C)** Voltage dependence of Best1 current in

HEK293T cells (~4.5 μM intracellular Ca²⁺) at the indicated intracellular GABA concentrations. In all cases, GABA was substituted for Cl⁻, and each current trace was normalized to the current measured at +100 mV. **(D)** Dependence of current reversal potential (E_{rev}) on intracellular GABA concentration ($n = 7$ to 14 for each point). Continuous curves are E_{rev} predicted by the Goldman-Hodgkin-Katz equation when GABA is as permeable as Cl⁻ ($P_{GABA}/P_{Cl} = 1$) and when GABA is not permeable at all ($P_{GABA}/P_{Cl} = 0$). **(E)** Relations between membrane potential and mean current density measured in HEK293T cells at the indicated intracellular Ca²⁺ concentrations ($n = 5$ to 7). Intracellular solution also contained 140 mM GABA. **(F)** Mean current densities recorded at -80 mV at different intracellular Ca²⁺ concentrations. Continuous curve indicates a fit of the Hill equation with an EC₅₀ of 150 nM and a Hill coefficient of 1.4.

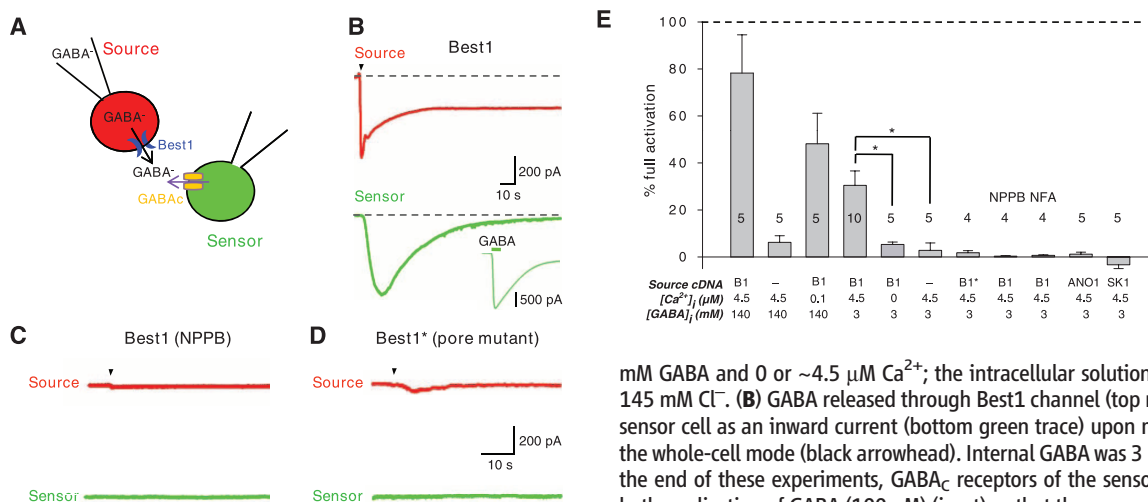


Fig. 2. GABA release via permeation through Best1 channels. **(A)** Schematic of two-cell bioassay. One HEK293T cell (Source) expressed Best1 and was labeled with dsRed; a second HEK cell (Sensor) expressed GABA_A receptors and was labeled with GFP. The intracellular solution for the source cell contained 3 or 140 mM GABA and 0 or ~4.5 μM Ca²⁺; the intracellular solution of the sensor cell contained 145 mM Cl⁻. **(B)** GABA released through Best1 channel (top red trace) was detected in the sensor cell as an inward current (bottom green trace) upon membrane breakthrough into the whole-cell mode (black arrowhead). Internal GABA was 3 mM, and Ca²⁺ was 4.5 μM. At the end of these experiments, GABA_A receptors of the sensor cell were fully activated by bath application of GABA (100 μM) (inset) so that the response to released GABA could be normalized according to the number of GABA receptors expressed in the sensor cell. Time-dependent reductions in sensor cell currents are due to desensitization of GABA receptors. **(C)** GABA release was blocked by NPPB (100 μM). **(D)** No GABA release was observed when the source cell expressed the pore mutant Best1-W93C. **(E)** GABA release measured in the indicated conditions, with values normalized as described above. NPPB and NFA were applied at 100 μM. SK1 is small conductance Ca²⁺-activated K⁺ channel. ANO1 is a recently characterized Ca²⁺-activated chloride channel. Numbers indicate the number of replicates in each condition, and asterisk indicates a significant difference ($P < 0.05$).

pounds on GABA_C receptors (fig. S10, a and b). Finally, GABA release could not be reconstituted by expression of other Ca²⁺-activated channels, such as the anion channel Ano1 (or TMEM-16A) or the potassium channel SK1 (Fig. 2E, and fig. S2) (24).

Best1 mediates tonic GABA release in cerebellum. We next asked whether treatments that interfere with Best1 function reduce tonic inhibition of cerebellar granule cells. We first used whole-cell patch-clamp recordings to measure the sustained Cl⁻ current associated with tonic inhibition (9) (Fig. 3A). Treatment of granule cells with the GABA_A receptor antagonist, GABA_A (SR95531 or SR; 10 μM), shifted this current by 35.7 ± 4.1 pA (*n* = 8), presumably by blocking GABA_A receptors activated by tonic GABA release (9). Treatment with NPPB also reduced the tonic current by 19.0 ± 2.5 pA (*n* = 8). This blockade of tonic GABA current by NPPB was not due to a direct action of this compound on granule cell GABA_A receptors (fig. S10, c and d). Two other anion channel blockers that block Best1, NFA and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid disodium salt hydrate (DIDS), similarly reduced tonic inhibitory currents in granule cells (fig. S11, b and g). The degree of inhibition

by these anion channel blockers ranged from about 50 to 75% of the total GABA_A-sensitive current.

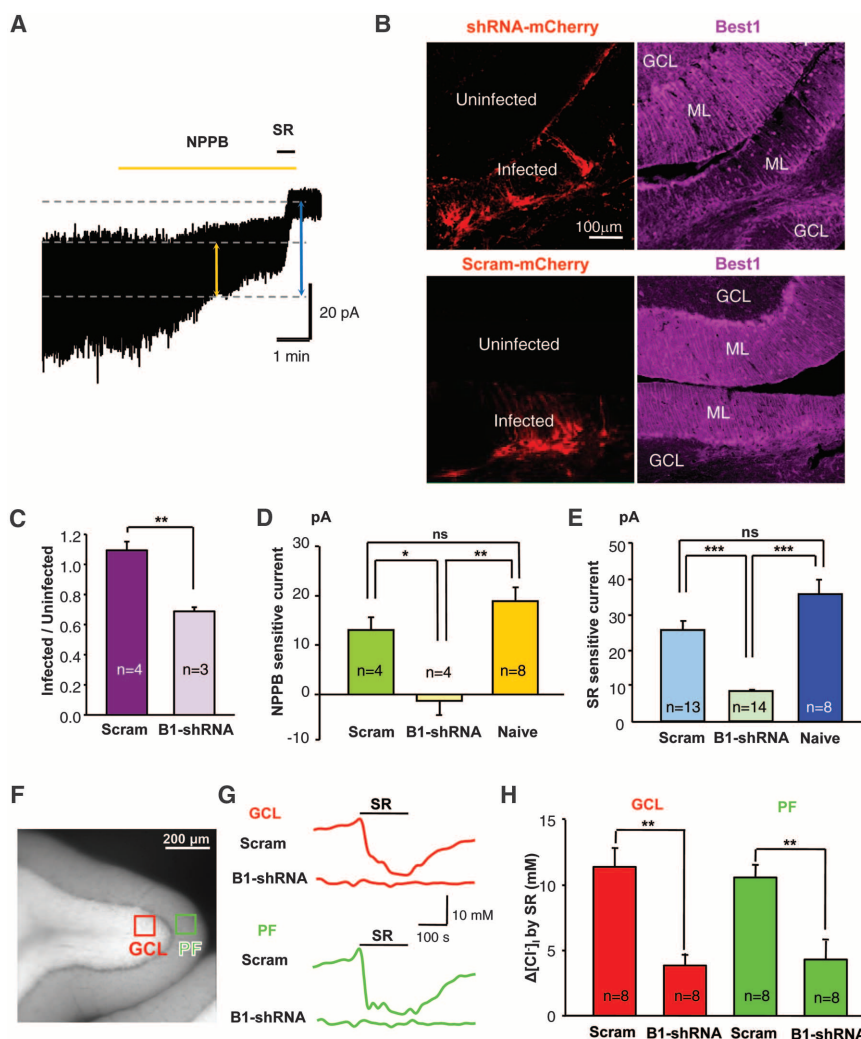
To more selectively inhibit Best1 function, we constructed a lentivirus carrying a small hairpin-forming interference RNA (shRNA) targeted against Best1 (12). This virus also contained DNA encoding a fluorescent marker, mCherry, that permitted visualization of the location and amount of viral infection. When Best1-shRNA lentivirus was injected into the cerebellar cortex of these mice, infected cells (red) were distributed widely in the molecular layer, as well as in the granule cell layer (Fig. 3B, top left). Quantification of Best1 immunoreactivity (Fig. 3C) indicated that regions expressing Best1-shRNA had significantly decreased (*P* < 0.003; *n* = 3) Best1 levels in comparison with nearby uninfected regions (Fig. 3B, top right), whereas there was no such difference in mice injected with the control scrambled-shRNA (Fig. 3B, bottom right, and 3C).

To examine the effects of Best1 deletion (knockdown) on tonic inhibition, cerebellar slices were prepared from mice injected with either Best1-shRNA or scrambled-shRNA viruses, or from uninjected (naïve) mice. The ability of NPPB to block tonic current was eliminated in granule cells from mice injected with Best1-shRNA and was present at con-

trol levels (naïve) in granule cells from mice injected with scrambled-shRNA (Fig. 3D). Likewise, the total GABA_A-sensitive tonic current was significantly decreased in granule cells from mice injected with Best1-shRNA lentivirus, relative to cells from naïve mice or mice injected with scrambled-shRNA virus (Fig. 3E). Total GABA_A-sensitive tonic current was reduced by ~75% by Best1 knockdown (Fig. 3E), consistent with the results obtained with channel-blocking drugs. The source of the remaining tonic inhibition requires further investigation.

We next visualized the spatial extent of tonic inhibition by using the optogenetic indicator, Clomeleon (25), to image Cl⁻ fluxes associated with tonic inhibition of granule cells (26). Cerebellar slices from a transgenic mouse line that expresses Clomeleon in cerebellar granule cells (Fig. 3F) were treated with 10 μM GABA_A to block tonic inhibition. This treatment markedly decreased intracellular Cl⁻ concentration ([Cl⁻]_i) in both granule cell bodies and their axons, parallel fibers. NPPB also lowered [Cl⁻]_i, although the yellow color of this drug prevented accurate determination of [Cl⁻]_i. To evaluate the role of Best1 in this Cl⁻ influx, we compared the GABA_A-induced changes in [Cl⁻]_i in slices from Clomeleon mice

Fig. 3. Tonic GABA current was reduced by attenuation of Best1. **(A)** Representative measurement of tonic current in a cerebellar granule cell held at -60 mV during treatment with NPPB (50 μM, yellow bar) and then GABA_A (SR, 10 μM, blue bar). Arrows indicate magnitude of tonic current blocked by each drug. **(B)** Fluorescence images of the granule cell layer (GCL) and the molecular layer (ML) in a histological section from a cerebellum injected with lentiviruses transducing either Best1-shRNA (top) or scrambled RNA (bottom). The viruses also transduced the fluorescent protein mCherry (left), to indicate infected cells. shRNA treatment reduced Best1 immunoreactivity (right) in the Best1-shRNA infected area but not in the area infected with scrambled shRNA. **(C)** Efficiency of Best1 knockdown by scrambled shRNA (Scram) and Best1 shRNA (B1-shRNA). Immunoreactivity was normalized to fluorescence values measured in uninfected regions. **(D)** Magnitude of NPPB-sensitive tonic current recorded in granule cells treated as indicated. **(E)** Magnitude of SR-sensitive tonic current recorded in the same conditions. **(F)** Clomeleon fluorescence in cerebellar slice from CLM1 transgenic mouse. [Cl⁻]_i was measured in granule cell layer (GCL, red) and parallel fibers (PF, green). **(G)** Time course of [Cl⁻]_i changes reported by Clomeleon during SR application in GCL (red) and PF (green). Clomeleon transgenic mice were injected with either scrambled shRNA (top traces) or Best1-shRNA (bottom traces). **(H)** SR-sensitive changes in [Cl⁻]_i measured in the indicated conditions. Asterisks indicate a significant difference determined by unpaired two-tailed *t* test (**P* < 0.05, ***P* < 0.01, ****P* < 0.001), while ns indicates a nonsignificant difference (*P* > 0.05).



in which either the Best1-shRNA or the scrambled-shRNA viruses were injected. For both granule cell bodies and parallel fibers, the $[Cl^-]_i$ changes produced by GABA_A were significantly decreased in slices from mice injected with Best1-shRNA in comparison with mice injected with scrambled shRNA (Fig. 3G). On average, Best1-shRNA treatment reduced SR-sensitive tonic inhibition by 60% or more (Fig. 3H), consistent with our electrophysiological results.

Glial cells contain GABA and express Best1 channel. Having identified a molecular mediator of tonic GABA release, we next used this information to identify the cellular source of the GABA release. We used immunohistochemical labeling to identify cells within the cerebellum that contain both Best1 and GABA (Fig. 4, A and B, and fig. S4). The specificity of the Best1 antibody was confirmed by Western blot analysis

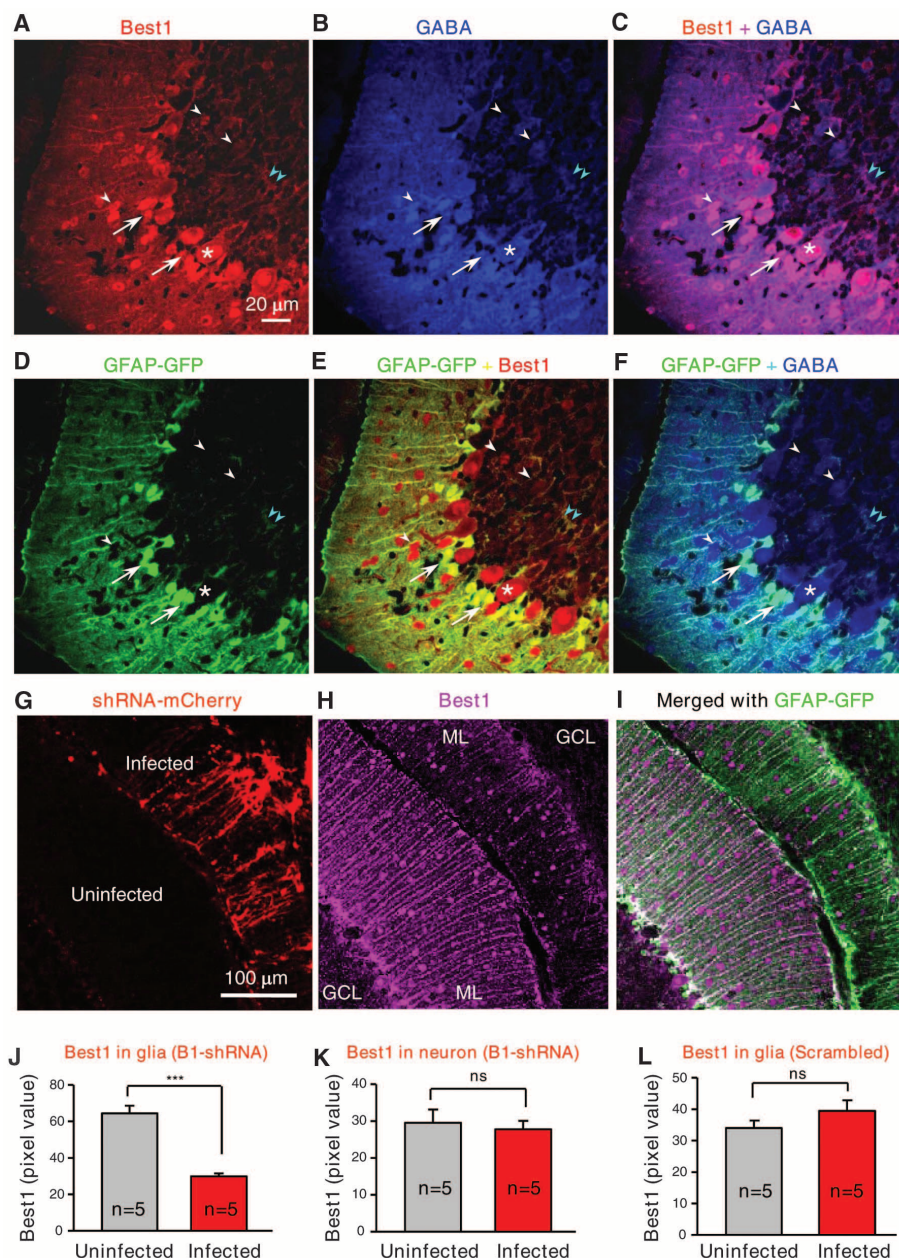
(fig. S3) (12). Best1 and GABA immunoreactivity colocalized in GABA-mediated Purkinje cells (asterisks) and interneurons (white arrowheads), but not in glutamatergic granule cells (Fig. 4C). Remarkably, both Best1 and GABA apparently were highly expressed in Bergmann glial cells (arrows), a point we examined further using GFAP-GFP transgenic mice, which have green fluorescent protein (GFP)-labeled astrocytes (Fig. 4D) (27). In these mice, GFP-positive Bergmann glial cells contained both Best1 (Fig. 4E) and GABA (Fig. 4F), even in Bergmann glial processes (Fig. 4, E and F, and fig. S4b) that closely interact with parallel fibers (28). In addition, both GABA and Best1 were expressed in lamellar astrocytes adjacent to granule cell bodies (pale blue arrowheads in Fig. 4, E and F, and fig. S4a).

We thus reexamined the effects of Best1-shRNA treatment to determine which cells were

affected by silencing of the Best1 gene (Fig. 4, G to I). To distinguish between neurons and glial cells, we again used GFAP-GFP transgenic mice and analyzed Best1 expression in cells that were GFP-positive (glia) or GFP-negative (neurons). Knockdown of Best1 was significant in glial cells (Fig. 4J) but not in neurons (Fig. 4K). This suggests that the U6 promoter drove shRNA expression more efficiently in glial cells than neurons, as reported for another promoter (29). There was no knockdown of Best1 in glia of mice injected with control, scrambled-shRNA (Fig. 4L).

Whole-cell patch-clamp recordings were used to determine whether Bergmann glial cells express functional, GABA-permeable Best1 channels. The internal solution contained either Cl^- or GABA as the predominant anions, plus 4.5 μM free Ca^{2+} to maximally activate Best1 channels. A Best1 blocker, NPPB (50 μM), reduced a tonic

Fig. 4. Presence of both GABA and Best1 in cerebellar glial cells. (A to F) Immunostaining for Best1 (A), GABA (B), and GFP (D) in sections from GFAP-GFP transgenic mice. The indicated image pairs are superimposed in (C), (E), and (F). Best1 and GABA were coexpressed in Purkinje cells (asterisk), interneurons (white arrowheads), Bergmann glia (arrows), and lamellar astrocytes (pale blue arrowheads), but not in granule cells. All GFP-positive astrocytes robustly expressed Best1 (E) and evinced GABA staining (F). (G to I) Images from the cerebellum of a GFAP-GFP mouse injected with Best1-shRNA lentivirus carrying mCherry (G). Best1 labeling (H) was merged with GFAP-GFP (I) (J to L) Quantification of Best1 expression in glia [GFP-positive pixels (J)]; in neurons [GFP-negative pixels (K)]; in infected and uninfected regions of cerebellar tissue from mice injected with Best1-shRNA, as well as in glia injected with scrambled-shRNA (L). Asterisks indicate a significant difference determined by unpaired two-tailed *t* test ($***P < 0.001$); ns indicates a nonsignificant difference ($P > 0.05$).



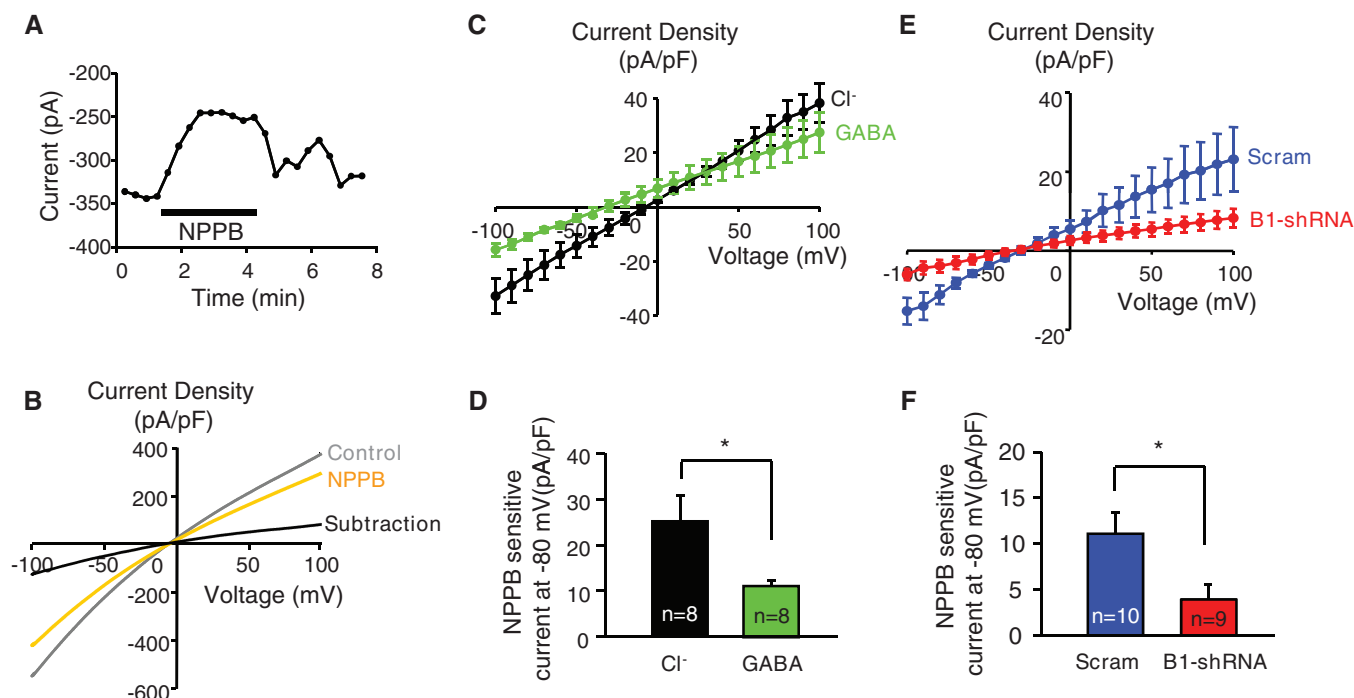
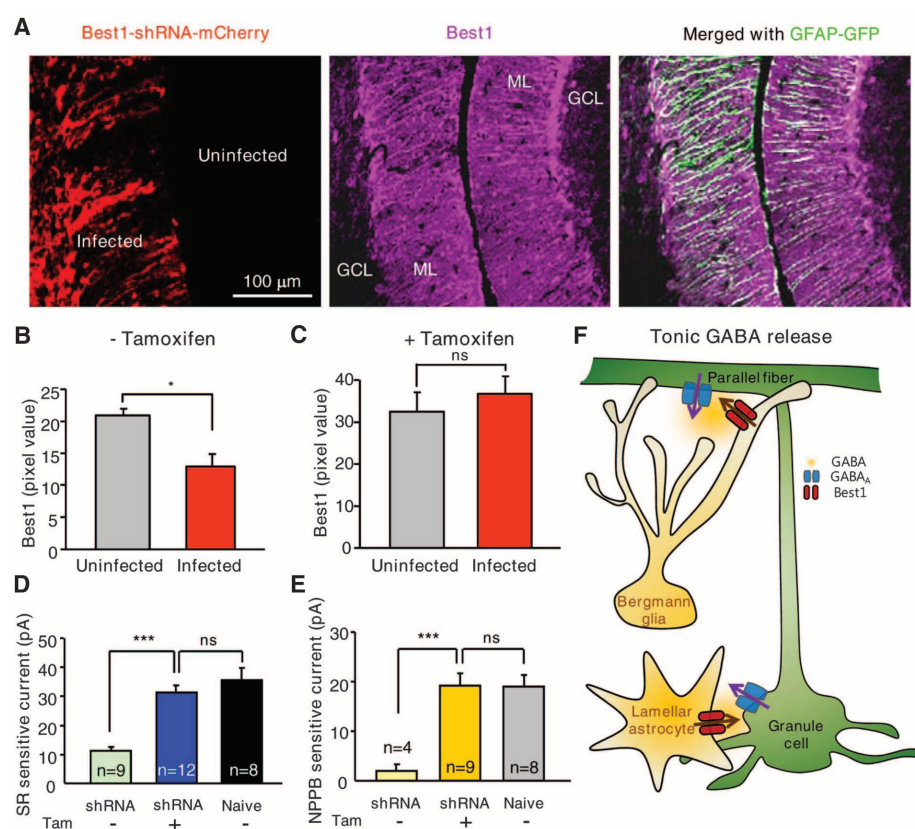


Fig. 5. Bergmann glia express GABA-permeable Best1 channels. **(A)** Current measured at -70 mV from a GFP-positive Bergmann glial cell in a cerebellar slice from a naïve GFAP-GFP mouse. Intracellular solution contained ~ 4.5 μ M Ca^{2+} and 150 mM Cl^- , as well as Cs^+ (146 mM) to block Ca^{2+} -dependent K^+ channels. Current was decreased by application of the anion channel blocker, NPPB (50 μ M, bar). **(B)** Voltage dependence of currents, determined as in Fig. 1, measured before and during NPPB application. The difference between these two relations (subtraction) represents NPPB-sensitive current. **(C)** Voltage dependence of

NPPB-sensitive current measured when internal solution contained Cl^- (150 mM) or GABA (140 mM). **(D)** Mean amplitude of NPPB-sensitive currents measured at -80 mV with either Cl^- or GABA inside the Bergmann glial cells. **(E)** Voltage dependence of NPPB-sensitive current measured in Bergmann glia from cerebella injected with scrambled shRNA (Scram) or Best1-shRNA (B1-shRNA). Internal solution contained 140 mM GABA. **(F)** Mean amplitude of NPPB-sensitive currents measured at -80 mV for the two conditions shown in (E). Asterisk indicates a significant difference determined by unpaired two-tailed t test ($P < 0.05$).

Fig. 6. Glia-specific rescue of Best1 restores tonic inhibition. **(A)** Viral transduction in cerebellum of tamoxifen-injected hGFAP-CreERT2 mice. (Left) Expression of mCherry, indicating virus-infected regions. (Middle) Best1 immunoreactivity in regions infected or uninfected by lentivirus. (Right) Merged images of mCherry, Best1, and GFP immunoreactivity. Note that, in this figure, the mCherry signal was electronically enhanced, relative to the images shown in other figures, because mCherry expression was reduced by tamoxifen treatment. **(B)** and **(C)** Quantification of Best1 immunoreactivity in hGFAP-CreERT2 mice injected with control solution (sunflower oil: – Tamoxifen) (B) or with tamoxifen (C). Asterisk indicates a significant difference ($P < 0.05$). **(D)** Tonic inhibition of granule cells measured in cerebellar slices from hGFAP-CreERT2 mice treated with tamoxifen and shRNA (shRNA +) or control mice (treated with sunflower oil: shRNA –) and compared with naïve, uninjected mice. Magnitude of tonic inhibition was determined from mean amplitude of current change produced by GABA_A application. **(E)** Magnitude of NPPB-sensitive current measured in the indicated conditions. **(F)** Model for tonic inhibition in the cerebellum: GABA is tonically released from glial cells via Best1 and activates GABA receptors on cell bodies and axons (parallel fibers) of granule cells. Asterisks indicate a significant difference determined by unpaired two-tailed t test (* $P < 0.05$, *** $P < 0.001$); ns indicates a nonsignificant difference ($P > 0.05$).



current in these cells (Fig. 5A). The voltage dependence of this current was determined by subtracting currents recorded during NPPB application from those measured before NPPB application (Fig. 5B). The NPPB-sensitive current measured with Cl^- as the predominant internal anion showed a reversal potential of -3.4 mV (Fig. 5C), which was similar to the reversal potential of $+1$ mV predicted for a Cl^- current and similar to Best1-expressing HEK293T cells (Fig. 1A). When GABA was the main internal anion (140 mM), the NPPB-sensitive current was inward at -80 mV, which indicated a significant efflux of GABA at this potential (Fig. 5, C and D). By measuring the reversal potential, we determined that the NPPB-sensitive anion current had a $P_{\text{GABA}}/P_{\text{Cl}}$ of 0.19 (Fig. 5C), similar to Best1-expressing HEK293T cells (Fig. 1D).

We further tested the function of Best1 in Bergmann glia by examining the effect of Best1-shRNA treatment on these cells (Fig. S8). Compared with scrambled-shRNA, treatment with Best1-shRNA decreased the NPPB-sensitive conductance (the slope of the current-voltage relations) in Bergmann glia without changing the reversal potential (Fig. 5E and Fig. S8, c and d). The effect on NPPB-sensitive GABA efflux was quantified by measuring current magnitude at -80 mV: GABA efflux at this potential was significantly smaller in Bergmann glia from mice injected with Best1-shRNA than with scrambled-shRNA (Fig. 5F, red) or from naïve mice (Fig. 5D, green).

Glial Best1 is responsible for tonic GABA release. To determine whether tonic GABA release is due to glial Best1, we used a molecular genetic strategy (30): Best1-shRNA was used to suppress Best1 expression throughout the cerebellar cortex, while selectively sparing Best1 expression in glia (30) (Fig. S5). In hGFAP-CreERT2 mice treated with Best1-shRNA, glial Best1 (Fig. 6, A and B) and GABAzine-sensitive tonic current (Fig. 6D and Fig. S9d) were significantly reduced, similar to those of wild-type mice (Fig. 3, C and E, and Fig. S9a). However, treating the hGFAP-CreERT2 mice with tamoxifen before Best1-shRNA injection reduced mCherry expression (Fig. S7), fully restored Best1 immunoreactivity (Fig. 6, A and C), and fully ($P < 0.001$) rescued GABAzine-sensitive currents to levels observed in naïve animals (Fig. 6D and Fig. S9). Similarly, NPPB-sensitive tonic currents were reduced by shRNA and fully rescued in tamoxifen-treated mice (Fig. 6E).

Discussion. We found that tonic release of the major inhibitory transmitter, GABA, is due to direct permeation of GABA through the anion channel, Best1, and that this release originates predominantly from glial cells. The mechanism underlying tonic GABA release has been difficult to elucidate because tonic GABA release exhibits several puzzling features that are quite different from those exhibited by conventional, phasic release of GABA. Our proposed mechanism can account for each of these properties: (i) the nonvesicular nature of tonic GABA release is consistent with a channel-mediated mechanism; (ii) the independence from neuronal activity can be explained by the glial

origins of tonic inhibition; and (iii) the apparent lack of dependence on external Ca^{2+} arises from substantial activation of Best1 at resting levels of intracellular Ca^{2+} (Fig. 1F), which leads to constitutive release of GABA at such intracellular Ca^{2+} levels (Fig. 2) (17, 31).

Our results provide several independent lines of evidence indicating that GABA is permeable through Best1. These include (i) shifts in current reversal potential when intracellular GABA concentration was varied (Fig. 1, C and D); (ii) current flow when GABA is the only permeant ion (Fig. S1); and (iii) Best1-dependent release of GABA from one cell onto another (Fig. 2). Collectively, these results demonstrate that GABA permeates through Best1. Although it has been proposed that Best1 has functions in addition to being an ion channel (17, 32, 33), our finding that a channel pore mutation blocks GABA release (Fig. 2D) indicates that GABA is released by direct permeation through the Best1 channel pore. We presume that tonic inhibition is caused by GABA (34); it remains a formal possibility that it is mediated by some other molecule that permeates Best1 and activates GABA receptors, such as taurine (Fig. S12).

To cause efflux through the Best1 channel, intracellular GABA concentration in Bergmann glial cells should be high enough to maintain the required electrochemical gradient. Bergmann glial cells show GABA immunoreactivity as intense as that observed in neighboring inhibitory neurons (Fig. 4B), which suggests a high GABA content, consistent with the intracellular GABA concentration of 3.5 mM reported in cultured astrocytes (35). With a submicromolar extracellular concentration of GABA (36), such high intracellular concentrations yield a positive equilibrium potential for GABA. Given the very negative resting-membrane potential of Bergmann glial cells, there is a strong electrochemical gradient to drive GABA efflux through the Best1 channel.

The GABA sensitivity [median effective concentration (EC_{50}) = 1.1 μM , Hill coefficient = 2.1] of GABA_C receptors (22) used in our two-cell biosensor microassay can be used to estimate a peak extracellular GABA concentration of 500 nM with 4.5 μM Ca^{2+} and 3 mM intracellular GABA concentration (Fig. 1E). At resting Ca^{2+} (100 nM), GABA efflux through Best1 is 31% of that measured at 4.5 μM Ca^{2+} (Fig. 1, E and F); this would yield an extracellular GABA concentration of 155 nM, which is remarkably close to the extracellular GABA concentration of 160 nM thought to be present during tonic inhibition (36).

Our work is consistent with previous indications that glia release GABA (37–39). Because Best1 is also volume-sensitive, our results provide a mechanism for the observation that swelling can trigger GABA release from glia (37). GABA usually is thought to be synthesized, contained, and released exclusively by neurons in adult brain. However, a handful of reports have suggested that astrocytes contain GABA (40), and our immunohistochemical data provide further support for this

idea. Although our work does not identify the source of GABA in Bergmann glia, it is known that GABA can be synthesized in glia via two pathways (41) and can be taken up into glia by GABA transporters (42).

The spatial organization of cerebellar glial cells is ideally suited to provide ambient GABA for tonic inhibition. In the type II glomerulus, the sheaths of lamellar astrocytes (43) are intimately associated with granule cell dendrites (44, 45). Such structures could permit the tonic inhibition of granule cell bodies and dendrites (9). Likewise, Bergmann glial cells tightly wrap around parallel fiber synapses (46, 47), providing a strategic location for tonic inhibition of parallel fibers (46). Our findings lead to a model for tonic GABA release (Fig. 6F): GABA in Bergmann glia permeates through the Best1 channel to activate GABA_A receptors on parallel fibers, while the same mechanisms allow lamellar astrocytes to tonically inhibit granule cell bodies and dendrites. By providing a source of GABA and by locating the molecular machinery for tonic GABA release near the granule cell structures, as well as by creating a restricted volume that allows tonic accumulation of GABA, it appears that glial cells are anatomically optimized for controlling electrical signaling in neighboring cerebellar neurons.

In conclusion, we have demonstrated an unprecedented mechanism for tonic GABA release through the bestrophin channel in cerebellar glial cells and propose a function for glia in modulating neuronal signaling via tonic inhibition.

References and Notes

- M. Farrant, Z. Nusser, *Nat. Rev. Neurosci.* **6**, 215 (2005).
- T. S. Otis, K. J. Staley, I. Mody, *Brain Res.* **545**, 142 (1991).
- M. Kaneda, M. Farrant, S. G. Cull-Candy, *J. Physiol.* **485**, 419 (1995).
- S. G. Brickley, S. G. Cull-Candy, M. Farrant, *J. Physiol.* **497**, 753 (1996).
- P. Chadderton, T. W. Margrie, M. Häusser, *Nature* **428**, 856 (2004).
- D. W. Cope *et al.*, *Nat. Med.* **15**, 1392 (2009).
- F. Jia *et al.*, *J. Neurophysiol.* **94**, 4491 (2005).
- V. B. Caraiacos *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 3662 (2004).
- D. J. Rossi, M. Hamann, D. Attwell, *J. Physiol.* **548**, 97 (2003).
- M. J. Wall, M. M. Usowicz, *Eur. J. Neurosci.* **9**, 533 (1997).
- K. Petrukhin *et al.*, *Nat. Genet.* **19**, 241 (1998).
- H. Park *et al.*, *J. Neurosci.* **29**, 13063 (2009).
- Z. Qu, H. C. Hartzell, *Am. J. Physiol. Cell Physiol.* **294**, C1371 (2008).
- K. E. O'Driscoll, N. Leblanc, W. J. Hatton, F. C. Britton, *Biochem. Biophys. Res. Commun.* **384**, 476 (2009).
- Materials and methods are available as supporting material on Science Online.
- Z. Qu, L. T. Chien, Y. Cui, H. C. Hartzell, *J. Neurosci.* **26**, 5411 (2006).
- H. C. Hartzell, Z. Qu, K. Yu, Q. Xiao, L. T. Chien, *Physiol. Rev.* **88**, 639 (2008).
- D. E. Clapham, *Cell* **131**, 1047 (2007).
- J. Glykys, I. Mody, *Neuron* **56**, 763 (2007).
- S. M. Jones, M. J. Palmer, *J. Neurophysiol.* **102**, 691 (2009).
- M. Chebib, *Clin. Exp. Pharmacol. Physiol.* **31**, 800 (2004).
- J. E. Carland *et al.*, *Neuropharmacology* **46**, 770 (2004).
- J. Amin, D. S. Weiss, *Receptors Channels* **2**, 227 (1994).
- C. T. Bond, J. Maylie, J. P. Adelman, *Curr. Opin. Neurobiol.* **15**, 305 (2005).
- T. Kuner, G. J. Augustine, *Neuron* **27**, 447 (2000).
- K. Berglund *et al.*, *Brain Cell Biol.* **36**, 101 (2008).
- M. Brenner, W. C. Kisileberth, Y. Su, F. Besnard, A. Messing, *J. Neurosci.* **14**, 1030 (1994).

28. T. Müller, H. Kettenmann, *Int. Rev. Neurobiol.* **38**, 341 (1995).
29. K. Takayama, T. Torashima, H. Horiuchi, H. Hirai, *Neurosci. Lett.* **443**, 7 (2008).
30. A. Ventura *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 10380 (2004).
31. S. Kirischuk, T. Möller, N. Voitenko, H. Kettenmann, A. Verkhratsky, *J. Neurosci.* **15**, 7861 (1995).
32. K. Yu, Z. Qu, Y. Cui, H. C. Hartzell, *Invest. Ophthalmol. Vis. Sci.* **48**, 4694 (2007).
33. K. Kunzelmann *et al.*, *Cell Calcium* **46**, 233 (2009).
34. P. Cavelier, M. Hamann, D. Rossi, P. Mobbs, D. Attwell, *Prog. Biophys. Mol. Biol.* **87**, 3 (2005).
35. J. Bardakdjian, M. Tardy, C. Pimoule, P. Gonnard, *Neurochem. Res.* **4**, 517 (1979).
36. V. Santhakumar, H. J. Hanchar, M. Wallner, R. W. Olsen, T. S. Otis, *J. Neurosci.* **26**, 3357 (2006).
37. A. S. Kozlov, M. C. Angulo, E. Audinat, S. Chrapak, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 10058 (2006).
38. S. Ochi *et al.*, *Glia* **9**, 188 (1993).
39. S. Oláh *et al.*, *Nature* **461**, 1278 (2009).
40. A. Blomqvist, J. Broman, *J. Neurocytol.* **17**, 629 (1988).
41. R. Martínez-Rodríguez *et al.*, *Cell Mol. Biol. (Noisy-le-grand)* **39**, 115 (1993).
42. A. Gadea, A. M. López-Colomé, *J. Neurosci. Res.* **63**, 461 (2001).
43. C. E. Ribak, W. M. Tong, N. C. Brecha, *Anat. Embryol. (Berlin)* **194**, 379 (1996).
44. V. Chan-Palay, S. L. Palay, *Z. Anat. Entwicklungsgesch.* **138**, 1 (1972).
45. D. M. Landis, L. A. Weinstein, J. J. Halperin, *Brain Res.* **284**, 231 (1983).
46. J. Grosche *et al.*, *Nat. Neurosci.* **2**, 139 (1999).
47. T. C. Bellamy, *Cerebellum* **5**, 116 (2006).
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Collaborative Non-Self Recognition System in S-RNase–Based Self-Incompatibility

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Self-incompatibility in flowering plants prevents inbreeding and promotes outcrossing to generate genetic diversity. In Solanaceae, a multiallelic gene, *S*-locus *F*-box (*SLF*), was previously shown to encode the pollen determinant in self-incompatibility. It was postulated that an SLF allelic product specifically detoxifies its non-self S-ribonucleases (S-RNases), allelic products of the pistil determinant, inside pollen tubes via the ubiquitin–26S-proteasome system, thereby allowing compatible pollinations. However, it remained puzzling how SLF, with much lower allelic sequence diversity than S-RNase, might have the capacity to recognize a large repertoire of non-self S-RNases. We used in vivo functional assays and protein interaction assays to show that in *Petunia*, at least three types of divergent SLF proteins function as the pollen determinant, each recognizing a subset of non-self S-RNases. Our findings reveal a collaborative non-self recognition system in plants.

Self-incompatibility (SI) is an intraspecific reproductive barrier adopted by angiosperms that allows the pistil to distinguish between self (genetically related) and non-self (genetically unrelated) pollen. In most cases, this self/non-self discrimination is controlled by male- and female-specificity determinants (pollen-S, style-S) encoded by multiallelic genes at the *S* locus (*1*, *2*). Self-incompatible species in Solanaceae, Rosaceae, and Plantaginaceae use extracellular S-RNase as style-S (*3*). If the *S* haplotype of pollen matches either *S* haplotype of the style, S-RNase exerts cytotoxicity inside the self-pollen tube to inhibit growth (*3*). Pollen-S was identified as an F-box protein, named S-locus F-box (SLF or SFB) (*4–6*), which may be a component of an SCF (Skp1–Cullin1–F-box) or SCF-like complex (*7*, *8*).

A protein degradation model was proposed to explain *S* haplotype-specific rejection of pollen tubes by S-RNase. It predicts that an SLF allelic variant specifically recognizes its non-self S-RNases and mediates their degradation by the ubiquitin–26S-proteasome system (*1*, *8*, *9*). This model can explain competitive interaction, where SI breaks down in heteroallelic pollen carrying two different pollen-S alleles (*10*, *11*). Each SLF allelic product in heteroallelic pollen mediates degradation of all S-RNases except its self S-RNase, and two different SLF allelic products together mediate the degradation of all S-RNases, rendering the pollen tube compatible with styles of any *S* genotype (*3*, *8*). Experiments designed on the basis of competitive interaction showed that *PiSLF*₂ (*S*₂ allele of *Petunia inflata* *SLF*) functions as pollen-S (*6*). When *PiSLF*₂ was introduced into *S*₁*S*₂ and *S*₂*S*₃ plants, it caused breakdown of SI in *S*₁ and *S*₃ pollen, but not in *S*₂ pollen, as predicted by competitive interaction (*6*).

Thus far, *PiSLF*₂ is the only *SLF* allele in *Petunia* shown to function as pollen-S (*6*, *9*). *SLF* shows much lower allelic sequence diversity than S-RNase, and nonsynonymous substitution rates of *SLFs* from *Antirrhinum*, *Petunia*, and *Prunus* are 0.01 to 0.11, whereas those for S-RNase are 0.14 to 0.51 (*12*, *13*). Given the large

number of *S* haplotypes within each species, it is puzzling how an SLF allelic product could recognize a large repertoire of highly divergent non-self S-RNases to allow cross-compatible pollinations. Moreover, phylogenetic studies of *SLF* and S-RNase in Solanaceae and Plantaginaceae showed no evidence of coevolution, with *SLF* having a much shorter evolutionary history (*12*), which is unexpected for the “male” and “female” genes encoding proteins directly involved in self/non-self discrimination during sexual reproduction. Here, we address the question of whether the previously identified SLF in *Petunia* is the only protein that constitutes the pollen determinant in SI.

Previously studied *SLF* is not the sole element of pollen-S. We first cloned four additional alleles of *Petunia* *SLF* from pollen cDNA of *S*₅, *S*₇, *S*₉, and *S*₁₁ homozygotes by 3′- and 5′-RACE (rapid amplification of cDNA ends) with the use of primers (table S1) designed on the basis of *PiSLF*₁, *PiSLF*₂, and *PiSLF*₃ sequences (*6*, *14*). The deduced amino acid sequences of all nine of the identified *SLF* alleles exhibited higher sequence similarities (86.4 to 100% identity) than the corresponding nine S-RNase alleles (40.1 to 79.4% identity) (fig. S1). Because the taxa with S-RNase-based SI have multiple *SLF*-like genes (*5*, *9*), we renamed *SLF* “type-1 *SLF*,” designating alleles as *S*_{*n*}-*SLF*₁, with *n* denoting the *S* haplotype.

A surprising finding from the sequence comparison was that the deduced amino acid sequence of *S*₇-*SLF*₁ is identical with that of *S*₁₉-*SLF*₁ (previously named *PaSLF*₁₉) (*11*), although the amino acid sequences of *S*₇- and *S*₁₉-RNase are 45% identical (fig. S1). Reciprocal pollinations between *S*₇ and *S*₁₉ homozygotes showed that all pollinations were compatible (Fig. 1A), confirming that *S*₇ and *S*₁₉ are distinct *S* haplotypes. The finding of the identical *SLF*₁ in two different *S* haplotypes raised the possibility that *SLF*₁ is not the sole element of pollen-S.

To address this possibility, we first examined whether *S*₅-, *S*₇- (= *S*₁₉-), *S*₉-, and *S*₁₁-*SLF*₁ function as pollen-S. We introduced each transgene construct (fig. S2) into appropriate *S* heterozygotes of *Petunia* (table S2) (*14*) and confirmed expression of each transgene in pollen by reverse transcription polymerase chain reaction (RT-PCR)

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(fig. S3). When an *S₇-SLF1* transgene was introduced into *S₅S₇*, *S₇S₁₁*, and *S₅S₁₉* plants, all transgenic plants remained self-incompatible (Fig. 1, B to D, and table S2), indicating that it did not cause competitive interaction either in homoallelic *S₇* and *S₁₉* pollen or in heteroallelic *S₅* and *S₁₁* pollen.

When the *S₇-SLF1* transgene was introduced into *S₇S₉* plants, however, they exhibited breakdown of SI (Fig. 1E), and reciprocal crosses with wild-type *S₇S₉* plants showed that *S₇-SLF1* caused breakdown of pollen, but not style, function in SI (Fig. 1, F and G). To determine whether breakdown of SI resulted from competitive interaction, we examined the inheritance of the *S₇-SLF1* transgene and progeny *S* genotypes from pollination of wild-type *S₇S₉* plants with pollen from an *S₇S₉/S₇-SLF1* plant. PCR analysis revealed that all 26 plants examined carried the transgene and were either *S₉S₉* (14 plants) or *S₇S₉* (12 plants) (Fig. 1H and table S3). The absence of an *S₇S₇* genotype suggested that only *S₉* pollen, but not *S₇* pollen, carrying the *S₇-SLF1* transgene became compatible with *S₇S₉* pistils. Competitive interaction was also observed when the *S₇-SLF1* transgene was expressed in *S₁₇* pollen (table S2); this confirms our previous conclusion that duplicated *S₇-SLF1* caused breakdown of SI in a naturally occurring self-compatible line of *Petunia* carrying an *S₁₇* haplotype (11). These results and those from other transgenic experiments (table S2) suggested that *S₇-SLF1* causes competitive inter-

action in only a subset of heteroallelic pollen (*S₉* and *S₁₇* pollen, but not *S₅* or *S₁₁* pollen) (Table 1).

Plants carrying *S₅-*, *S₉-* or *S₁₁-SLF1* transgenes were similarly analyzed (tables S2 and S3); their effects on SI behavior are summarized in Table 1. As with *S₇-SLF1*, each caused competitive interaction only in pollen of a subset of non-self *S* haplotypes. We further examined the function of previously identified *S₂-SLF1* and *S₃-SLF1* of *P. inflata* (14). Consistent with our previous finding (6, 9), *S₂-SLF1* caused competitive interaction in *S₃* pollen. However, neither *S₃-SLF1* nor *S₃-SLF1:GFP* caused competitive interaction in *S₂* pollen, even though RT-PCR showed that both were expressed in pollen; the level of the green fluorescent protein (GFP) fusion protein *S₃-SLF1:GFP* was comparable to that of *S₂-SLF1:GFP* produced in a previously generated *S₂S₃* transgenic plant, where it caused breakdown of SI (9) (fig. S4, and tables S2 and S3).

All in vivo functional assays suggested that each *SLF1* allele elicits competitive interaction in pollen of a subset of its non-self *S* haplotypes. On the basis of the protein degradation model, this would suggest that for each *S* haplotype, an *SLF1* allelic variant only recognizes and interacts with a subset of its non-self S-RNases and mediates their degradation. This raised the question of what other protein(s) encoded at the *S* locus might be required.

Pollen-S comprises multiple types of *SLF* genes. In *P. inflata*, several *SLF*-like genes were found tightly linked to the *S* locus (9). *PiSLFLb-S₂*,

PiSLFLc-S₁, and *PiSLFLd-S₂* were studied, but these did not cause competitive interaction in their respective heteroallelic pollen (9). We hypothesized that these and additional *SLF*-like genes might be elements of pollen-S, but like *SLF1*, their function would only be revealed in pollen of appropriate *S* haplotypes. To test this hypothesis, we designed PCR primers (table S1) based on previously characterized *SLF*-like genes, and extensively amplified *SLF*-like cDNA fragments by 3'- and 5'-RACE from pollen of *S₅*, *S₇*, *S₉*, *S₁₁*, *S₁₇*, and *S₁₉* haplotypes (14).

We identified 30 *SLF*-like sequences and classified all except *S₅-FBX* into five subgroups (Fig. 2), named type-2 to type-6 *SLF*. Using the same nomenclature as for type-1 *SLF*, we designated alleles *S_i-SLFx*. The previously identified *PiSLFLc-S₁* and *PiSLFLd-S₂* (9) were renamed *S₇-SLF2* and *S₂-SLF3*. We identified the alleles of all *SLF* types from these six *S* haplotypes, except that of type-2 *SLF* from the *S₉* haplotype (fig. S5). Sequence identities between allelic variants of each type were 72.2 to 87.5% (type 2), 70.3 to 99.0% (type 3), 89.6 to 96.5% (type 4), 96.8 to 98.2% (type 5), and 93.4 to 95.9% (type 6). In contrast, identities between different types of *SLF* were only ~50% (fig. S6).

All six types of *SLF* genes showed similar developmentally regulated, male reproductive organ-specific expression profiles: Transcripts increased during anther development, peaked before anthesis, and declined in mature pollen and pollen

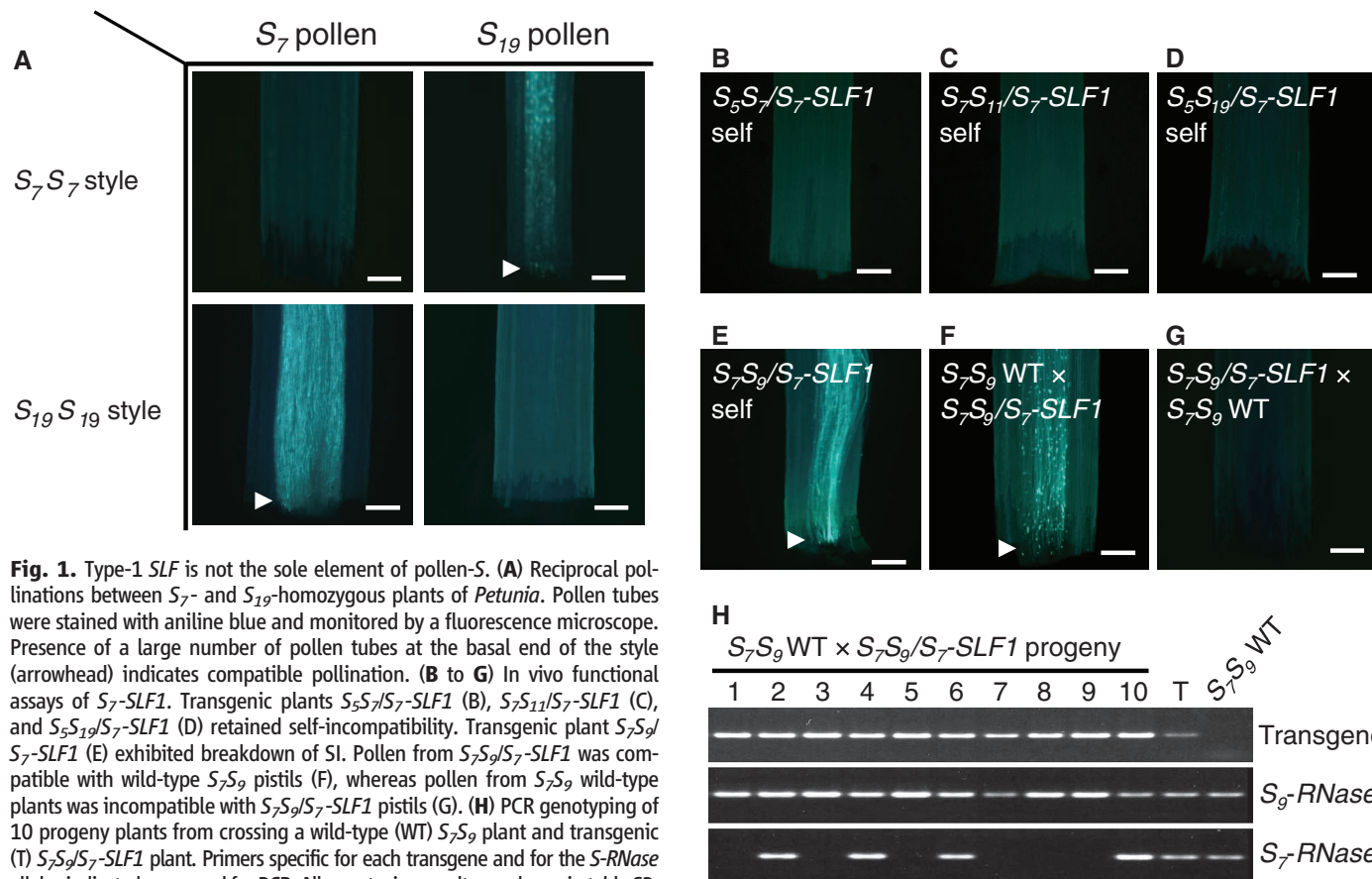


Fig. 1. Type-1 *SLF* is not the sole element of pollen-S. (A) Reciprocal pollinations between *S₇-* and *S₁₉-*homozygous plants of *Petunia*. Pollen tubes were stained with aniline blue and monitored by a fluorescence microscope. Presence of a large number of pollen tubes at the basal end of the style (arrowhead) indicates compatible pollination. (B to G) In vivo functional assays of *S₇-SLF1*. Transgenic plants *S₅S₇/S₇-SLF1* (B), *S₇S₁₁/S₇-SLF1* (C), and *S₅S₁₉/S₇-SLF1* (D) retained self-incompatibility. Transgenic plant *S₇S₉/S₇-SLF1* (E) exhibited breakdown of SI. Pollen from *S₇S₉/S₇-SLF1* was compatible with wild-type *S₇S₉* pistils (F), whereas pollen from *S₇S₉* wild-type plants was incompatible with *S₇S₉/S₇-SLF1* pistils (G). (H) PCR genotyping of 10 progeny plants from crossing a wild-type (WT) *S₇S₉* plant and transgenic (T) *S₇S₉/S₇-SLF1* plant. Primers specific for each transgene and for the *S-RNase* alleles indicated were used for PCR. All genotyping results are shown in table S3.

tubes, although the timing of increase and rate of decline differed slightly among members (fig. S7). Examination of 43 plants segregating for *S*₅, *S*₇, *S*₉, and *S*₁₁ haplotypes and 33 plants segregating for *S*₁₇ and *S*₁₉ haplotypes showed no recombination between any allele of each type of *SLF* and *S-RNase* (fig. S8), which suggests that all six types of *SLF* genes are linked to the *S* locus (within 1 cM). These properties implied that the additional five types of *SLF* genes could potentially be elements of pollen-*S*.

We examined the *in vivo* function of the *S*₅, *S*₇, and *S*₁₁ alleles of type-2 *SLF* and the *S*₇ and *S*₁₁ alleles of type-3 *SLF* (fig. S2) (14); the effects on SI behavior are summarized in Table 1 (see also tables S2 and S3). All except *S*₇-*SLF3* caused competitive interaction; however, they affected different subsets of non-self *S* haplotypes of pollen. For the six *S* haplotypes of pollen examined, competitive interaction was observed in all but *S*₅ pollen. The findings that none of the three types of *SLF* genes of either *S*₇ or *S*₁₁ haplotype caused competitive interaction in *S*₅ pollen suggest that some other type(s) of *SLF* in *S*₇ and *S*₁₁ haplotypes mediate detoxification of *S*₅-RNase, allowing them to be compatible with *S*₅ styles. Thus, our transformation experiments suggested that at least three types of *SLF* proteins function as elements of pollen-*S*.

Each SLF type interacts with only a subset of S-RNase. We have modified the protein degradation model to consider all types of *SLFs*. For example, *S*₉ pollen is incompatible with *S*₉ styles because none of the multiple types of *SLFs* produced in *S*₉ pollen recognizes *S*₉-RNase, whereas *S*₉ pollen expressing *S*₇-*SLF2* is compatible with *S*₉ styles, because *S*₇-*SLF2* recognizes *S*₉-RNase and mediates its degradation. To test the validity of this model, we performed co-immunoprecipitation experiments. We expressed FLAG-tagged *S*₇-*SLF2* (fig. S2) in pollen of an *S*₅*S*₁₁ heterozygote, and found that it, like *S*₇-*SLF2*,

elicited competitive interaction in *S*₁₁ pollen (tables S2 and S3), suggesting that fusion of the FLAG tag did not affect SI function of *S*₇-*SLF2*.

Pollen extracts from *S*₅*S*₁₁/*FLAG*:*S*₇-*SLF2* and *S*₅*S*₁₁ wild-type plants were mixed with style extracts from various *S* homozygotes and immunoprecipitated with immobilized antibody to FLAG. When style extracts of *S*₉ and *S*₁₁ homozygotes were separately mixed with pollen extracts, strong bands of *S*₉-RNase and *S*₁₁-RNase were detected in the immunoprecipitates from transgenic pollen extracts, but only a faint *S*₁₁-RNase band of background level was detected in wild-type pollen extracts. This finding suggested that *S*₇-*SLF2* interacts strongly with *S*₉-RNase and *S*₁₁-RNase (Fig. 3, A and B). When style extracts of *S*₇ and *S*₅ homozygotes were mixed with pollen extracts of the transgenic plants, a faint *S*₇-RNase band of background level and no *S*₅-RNase band were detected (Fig. 3, C and D). These results suggested that the ability of the *S*₇-*SLF2* transgene to cause competitive interaction in *S*₉ and *S*₁₁ pollen, but not in *S*₅ and *S*₇ pollen, is due to specific interactions between *S*₇-*SLF2* and *S*₉- and *S*₁₁-RNases.

A collaborative recognition model. On the basis of this study, we propose a “collaborative non-self recognition” model for S-RNase-based SI (Fig. 4). In *Petunia*, pollen-*S* comprises multiple types of *SLF* genes. Within an *S* haplotype, the product of each type of *SLF* interacts with a subset of non-self S-RNases, and the products of multiple types, including yet uncharacterized ones, are required for the entire suite of non-self S-RNases to be collectively recognized and detoxified. This relationship between style-*S* and pollen-*S* is radically different from that of the SI systems possessed by the Brassicaceae and Papaveraceae, where both style-*S* and pollen-*S* are single genes, and self-interaction between style-*S* and pollen-*S* triggers SI responses (1, 15, 16).

In plants possessing S-RNase-based SI, increasing the repertoire of *SLF* genes that con-

stitute pollen-*S* would be advantageous, as this would increase the number of potential mating partners by allowing pollen to recognize and inactivate more non-self S-RNases, whereas an increase in diversity of the *S-RNase* gene would have the opposite effect by allowing new S-RNases to escape detoxification by the existing repertoire of *SLF* proteins. Other self-incompatible species in Solanaceae, Plantaginaceae, and Rosaceae also have a single *S-RNase* gene and multiple *SLF/SFB* genes located at their *S* loci (5, 17–20). For example, 10 and 12 *SLF/SFB*-like genes are linked to the *S* loci of *Nicotiana glauca* (Solanaceae) and apple (*Malus × domestica*, Rosaceae), respectively (18, 20). It would be interesting to determine whether any of these *S*-linked *F-box* genes function as elements of pollen-*S*.

The collaborative non-self recognition model can be further tested through analysis of loss-of-

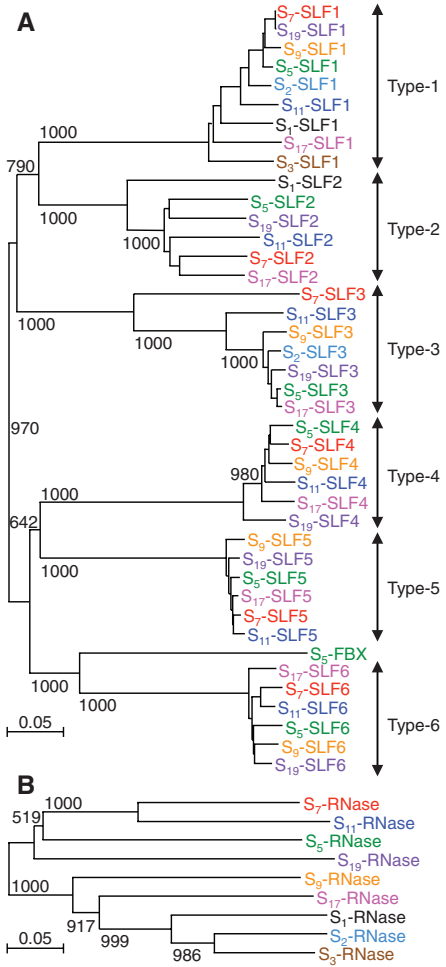


Fig. 2. Genealogies of SLF and S-RNase from *Petunia*. (A and B) Phylogenetic trees of deduced amino acid sequences of *SLF* genes (A) and *S-RNase* genes (B) were created with the bootstrap neighbor-joining algorithm (14). Both trees are shown to the same scale; the bar for each tree indicates the number of amino acid substitutions per site. Numbers on the branches indicate bootstrap values when the number of bootstrap trials is 1000. *S*₁₁, *S*₂, and *S*₃ alleles of these genes are from *P. inflata* (6), *S*₁₇ and *S*₁₉ alleles are from *P. axillaris* (11); all others are from *P. hybrida*.

Table 1. Effects of *SLF* transgenes on SI behavior of transgenic pollen. Abbreviations: +, breakdown of SI caused by competitive interaction; –, no breakdown of SI; (homo), homoallelic relationship between the *S* haplotype of the transgene and that of pollen.

Transgene	Haplotype of transgenic pollen					
Type-1 <i>SLF</i>						
	<i>S</i> ₅	<i>S</i> ₇	<i>S</i> ₉	<i>S</i> ₁₁	<i>S</i> ₁₇	<i>S</i> ₁₉
<i>S</i> ₅ - <i>SLF1</i>	– (homo)	–	+	–	+	–
<i>S</i> ₇ - <i>SLF1</i>	–	– (homo)	+	–	+	–
<i>S</i> ₉ - <i>SLF1</i>	–	–	– (homo)	–	+	–
<i>S</i> ₁₁ - <i>SLF1</i>	–	–	–	– (homo)	+	–
Type-2 <i>SLF</i>						
	<i>S</i> ₅	<i>S</i> ₇	<i>S</i> ₉	<i>S</i> ₁₁	<i>S</i> ₁₇	<i>S</i> ₁₉
<i>S</i> ₅ - <i>SLF2</i>	– (homo)	–	+	+	–	–
<i>S</i> ₇ - <i>SLF2</i>	–	– (homo)	+	+	–	+
<i>S</i> ₁₁ - <i>SLF2</i>	–	–	+	– (homo)	–	–
Type-3 <i>SLF</i>						
	<i>S</i> ₅	<i>S</i> ₇	<i>S</i> ₉	<i>S</i> ₁₁	<i>S</i> ₁₇	<i>S</i> ₁₉
<i>S</i> ₇ - <i>SLF3</i>	–	– (homo)	–	–	–	–
<i>S</i> ₁₁ - <i>SLF3</i>	–	+	–	– (homo)	–	–

function mutants of one or more types of *SLF* genes. So far, one such naturally occurring self-compatible mutant of *Petunia* has been identified, and the breakdown in SI is attributed to competitive interaction caused by duplication of an *SLF1* (11). Our previous model invoking a single *SLF* gene for pollen-*S* predicted that loss of function of pollen-*S* would be lethal, because such mutant pollen would be incapable of detoxifying any non-

self *S*-RNases and would thus be incompatible with styles of any *S* haplotype (3). However, according to our new model, loss of function in a single type of *SLF* would be lethal only for the haplotypes whose *S*-RNases are recognized by the type of *SLF* affected by mutation. Note that the SI behavior of a self-compatible cultivar, Osa-Nijisseiki, of Japanese pear (*Pyrus pyrifolia*, Rosaceae) is consistent with this prediction. This mutant has

a 236-kb deletion in the *S* locus of the *S*₄ haplotype, including an *SLF*-like gene, *S*₄*F-box0* (21), and its *S*₄ pollen is incompatible with *S*₁ styles, although it is compatible with the styles of other non-self *S* haplotypes tested (22). Our model would predict that *S*₄*F-box0* is an element of pollen-*S* in *Pyrus* that specifically recognizes *S*₁-RNase.

SI has been compared with adaptive immunity in vertebrates (23, 24). The use of products of multiple polymorphic *SLF* genes to collectively recognize a suite of non-self *S*-RNases in order to mediate their degradation is conceptually similar to specific recognition of foreign antigens by a large repertoire of T cell receptors. Our findings provide fertile ground for studying the emergence of multiple types of *SLF* genes, their coevolution with a single *S*-RNase gene, and the biochemical basis that allows a particular type of *SLF* to recognize certain non-self *S*-RNases but not others or self *S*-RNase.

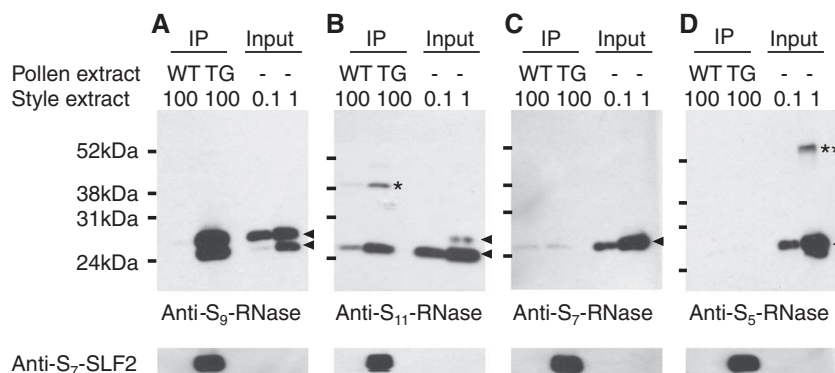


Fig. 3. Analysis of interactions between *S*₇-SLF2 and four allelic variants of *S*-RNase [(A) *S*₉-RNase, (B) *S*₁₁-RNase, (C) *S*₇-RNase, (D) *S*₅-RNase] by coimmunoprecipitation. Pollen extracts from a transgenic plant (TG) expressing *FLAG:S*₇-SLF2 or from a wild-type plant (WT) were mixed with style extracts from (A) *S*₉, (B) *S*₁₁, (C) *S*₇, and (D) *S*₅ homozygotes and then immunoprecipitated using an antibody to FLAG. Immunoprecipitates (IP) were analyzed by immunoblotting with antibodies specific for each *S*-RNase; 0.1% and 1% of the style extracts (input) used for the immunoprecipitation were loaded as controls. Immunoprecipitated FLAG-*S*₇-SLF2 was also detected by an antibody specific for *S*₇-SLF2 using the same membranes after detection of *S*-RNases (lower panels). In each upper panel, arrowheads indicate *S*-RNase bands. The double bands in (A) and (B) represent differentially glycosylated forms of *S*-RNase, as a band with smaller molecular mass was observed when each *S*-RNase was subjected to glycosidase treatment. The asterisk in (B) indicates an unidentified *S*₁₁-RNase derivative. The double asterisk in (D) indicates a dimeric form of *S*₅-RNase, as it was not observed in higher concentration (e.g., 50 mM) of dithiothreitol than was used in the SDS-polyacrylamide gel electrophoresis shown.

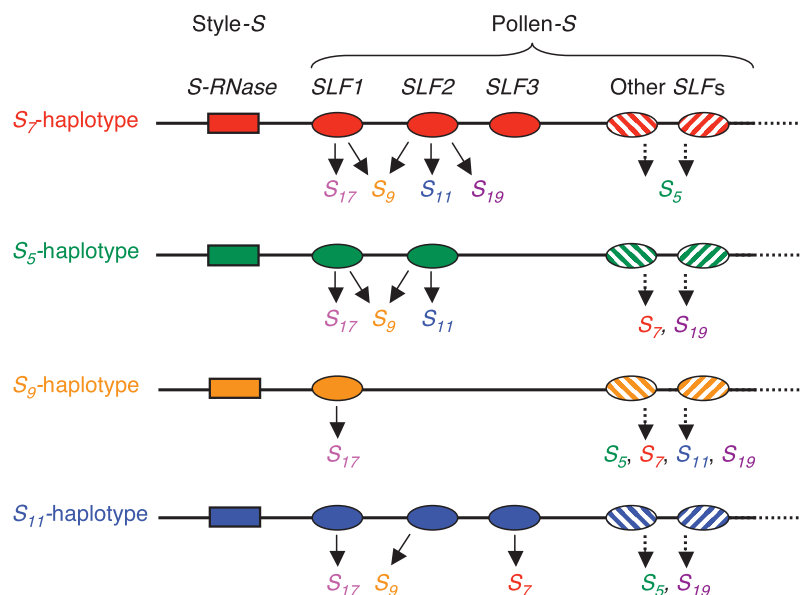


Fig. 4. Collaborative non-self recognition model for SI in *Petunia*. The single *S*-RNase gene constituting style-*S* and the multiple *SLFs* constituting pollen-*S* are depicted as boxes and ovals, respectively. The locations of these *SLFs* relative to *S*-RNase are as yet undetermined, but for convenience they are placed in a cluster. For each *S* haplotype, the *SLFs* whose products are responsible for detoxifying one or more of the five allelic variants of *S*-RNase tested are connected by solid arrows with their target alleles. For each *S* haplotype, products of one or more Other *SLFs* (including *SLF3s* of *S*₅ and *S*₉ haplotypes) are predicted to target products of the remaining alleles of *S*-RNase connected by broken arrows.

References and Notes

1. S. Takayama, A. Isogai, *Annu. Rev. Plant Biol.* **56**, 467 (2005).
2. V. E. Franklin-Tong, *Self-Incompatibility in Flowering Plants: Evolution, Diversity, and Mechanisms* (Springer, Heidelberg, 2008).
3. Z. H. Hua, A. Fields, T. H. Kao, *Mol. Plant* **1**, 575 (2008).
4. Z. Lai et al., *Plant Mol. Biol.* **50**, 29 (2002).
5. T. Entani et al., *Genes Cells* **8**, 203 (2003).
6. P. Sijacic et al., *Nature* **429**, 302 (2004).
7. H. Qiao et al., *Plant Cell* **16**, 582 (2004).
8. Z. Hua, T. H. Kao, *Plant Cell* **18**, 2531 (2006).
9. Z. Hua, X. Meng, T. H. Kao, *Plant Cell* **19**, 3593 (2007).
10. T. Entani et al., *Biosci. Biotechnol. Biochem.* **63**, 1882 (1999).
11. T. Tsukamoto, T. Ando, H. Watanabe, E. Marchesi, T. H. Kao, *Plant Mol. Biol.* **57**, 141 (2005).
12. E. Newbigin, T. Paape, J. R. Kohn, *Plant Cell* **20**, 2286 (2008).
13. B. McClure, *J. Exp. Bot.* **60**, 1069 (2009).
14. See supporting material on Science Online.
15. S. Takayama et al., *Nature* **413**, 534 (2001).
16. M. J. Wheeler et al., *Nature* **459**, 992 (2009).
17. J. Zhou et al., *Sex. Plant Reprod.* **16**, 165 (2003).
18. D. Wheeler, E. Newbigin, *Genetics* **177**, 2171 (2007).
19. H. Sassa et al., *Genetics* **175**, 1869 (2007).
20. M. Minamikawa et al., *Plant Mol. Biol.* **74**, 143 (2010).
21. K. Okada et al., *Plant Mol. Biol.* **66**, 389 (2008).
22. K. Okada et al., *J. Jpn. Soc. Hort. Sci.* **73**, 524 (2004).
23. F. M. Burnet, *Nature* **232**, 230 (1971).
24. T. Boehm, *Cell* **125**, 845 (2006).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6005/796/DC1
Materials and Methods
Figs. S1 to S8
Tables S1 to S3
References

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The Detection of a Population of Submillimeter-Bright, Strongly Lensed Galaxies

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Gravitational lensing is a powerful astrophysical and cosmological probe and is particularly valuable at submillimeter wavelengths for the study of the statistical and individual properties of dusty star-forming galaxies. However, the identification of gravitational lenses is often time-intensive, involving the sifting of large volumes of imaging or spectroscopic data to find few candidates. We used early data from the Herschel Astrophysical Terahertz Large Area Survey to demonstrate that wide-area submillimeter surveys can simply and easily detect strong gravitational lensing events, with close to 100% efficiency.

When the light from a distant galaxy is deflected by a foreground mass—commonly a massive elliptical galaxy or galaxy cluster or group—its angular size and brightness are increased, and multiple images of the same source may form. This phenomenon is

commonly known as gravitational lensing (1) and can be exploited in the study of high-redshift galaxy structures down to scales difficult (if not impossible) to probe with the largest telescopes at present (2–4) and to detect intrinsically faint objects. Surveys conducted at submillimeter wave-

lengths can particularly benefit from gravitational lensing because submillimeter telescopes have limited spatial resolution and consequently high source confusion, which makes it difficult to directly probe the populations responsible for the bulk of background submillimeter emission (5, 6). In addition, galaxies detected in blank-field submillimeter surveys generally suffer severe dust obscuration and are therefore challenging to detect and study at optical and near-infrared (NIR) wavelengths. By alleviating the photon starvation, gravitational lensing facilitates follow-up observations of galaxies obscured by dust and in particular the determination of their redshift (7). Previous submillimeter searches for highly magnified background galaxies have predominantly targeted galaxy cluster fields (8). In fact, a blind search for submillimeter lensing events requires a large area because of their rarity and sub-arcseconds angular resolutions to reveal multiple images of the same background galaxies.

Although the first requirement has recently been fulfilled, thanks to the advent of the South Pole Telescope (SPT) (9) and the Herschel Space Observatory (Herschel) (10), the second is still the prerogative of ground-based interferometric facilities, such as the Submillimeter Array (SMA) and the IRAM Plateau de Bure Interferometer (PdBI), which because of their small instantaneous field of view are aimed at follow-up observations rather than large-area survey campaigns. Nevertheless, several authors (11–14) have suggested that a simple selection in flux density, rather than surveys for multiply imaged sources, can be used to easily and efficiently select samples of strongly gravitationally lensed galaxies in wide-area submillimeter and millimeter surveys. The explanation for this lies in the steepness of

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the number counts (the number of galaxies at a given brightness) of dust-obscured star-forming galaxies, which are usually referred to as submillimeter galaxies (SMGs) (15). Because of that, even a small number of highly magnified SMGs can substantially affect the shape of the bright end of the submillimeter source counts enhancing the number of SMGs seen at bright flux densities than would be expected on the basis of our knowledge of the unlensed SMG population (Fig. 1). Furthermore, the frequency of lensing events is relatively high in the submillimeter (11) because SMGs are typically at high redshift ($z > \sim 1$) (16), and this increases the probability that a SMG is in alignment with, and therefore lensed by, a foreground galaxy. Other important contributors to the bright tail of the submillimeter counts are low-redshift ($z \leq 0.1$) spiral and starburst galaxies (17) and higher redshift radio-bright Active Galactic Nuclei (AGNs) (18); however, both of these are easily identified, and therefore removed, in relatively shallow optical and radio surveys. Therefore, flux-density-limited submillimeter surveys could provide a sample of lens candidates from which contaminants can be readily removed, leaving a high fraction (close to 100%) of gravitational lens systems (Fig. 1). Because this selection of lens candidates relies only on the properties of the background source (its flux density), it can probe a wide range of lens properties (such as redshifts and masses) and thus provide a valuable sample for studying the elliptical properties of lensing galaxies (19) as well as investigating the detailed properties of the lensed SMGs.

The submillimeter lens candidate selection at work. Although the approach presented above may be more efficient and vastly more time-effective than those exploited so far in the radio (20) or the optical (21, 22), at least several tens of square degrees (deg^2) of the sky must be observed in the submillimeter to produce a statistically significant sample of strongly lensed objects and a minimal contamination from unlensed galaxies. This is because the surface density of lensed submillimeter galaxies is predicted to be lower than $\sim 0.5 \text{ deg}^{-2}$ for flux densities above 100 mJy at 500 μm (Fig. 1). Submillimeter surveys conducted before the advent of Herschel were either limited to small areas of the sky (15, 23) or were severely affected by source confusion due to poor spatial resolution (24). Therefore, no previous test of this selection method has been performed, although the SPT has recently mapped an area of more than 80 deg^2 at millimeter wavelengths (9) and found an “excess” of sources that could be accounted for by a population of gravitationally lensed objects.

The Herschel Astrophysical Terahertz Large Area Survey (H-ATLAS) (25) represents the largest-area submillimeter survey being currently undertaken by Herschel. H-ATLAS uses the Spectral and Photometric Imaging Receiver (SPIRE) (26) and the Photodetector Array Camera and Spectrometer (PACS) (27, 28) instruments and, when completed, will cover $\sim 550 \text{ deg}^2$ of the sky

from 100 to 500 μm . H-ATLAS has been designed to observe areas of the sky with previously existing multiple-wavelength data: Galaxy Evolution Explorer (GALEX) ultraviolet (UV) data, Sloan Digital Sky Survey (SDSS) optical imaging and spectroscopy, NIR data from the UK Infrared Telescope (UKIRT) Infrared Deep Sky Survey (UKIDSS) Large Area Survey (LAS), spectra from the Galaxy And Mass Assembly (GAMA) (29) project, radio-imaging data from the Faint Images of the Radio Sky at Twenty-cm (FIRST) survey and the NRAO Very Large Array Sky Survey (NVSS). The first 14.4 deg^2 of the survey, centered on J2000 RA 09:05:30.0 DEC 00:30:00.0 and covering $\sim 3\%$ of the total area, was observed in November 2009 as part of the Herschel Science Demonstration Phase (SDP). The results were a catalog of ~ 6600 sources (30), with a significance $> 5\sigma$, in at least one SPIRE waveband, where the noise (σ) includes both instrumental and source confusion noise and corresponds to ~ 7 to 9 mJy/beam.

Fig. 1. Selection of gravitational lenses at submillimeter wavelengths. The 500- μm source counts consist of three different populations (14): high-redshift SMGs; lower redshift late type (starburst plus normal spiral) galaxies; and radio sources powered by active galactic nuclei. Strongly lensed SMGs dominate over unlensed SMGs at very bright fluxes, where the count of unlensed SMGs falls off dramatically (yellow shaded region). The data points are from H-ATLAS (31).

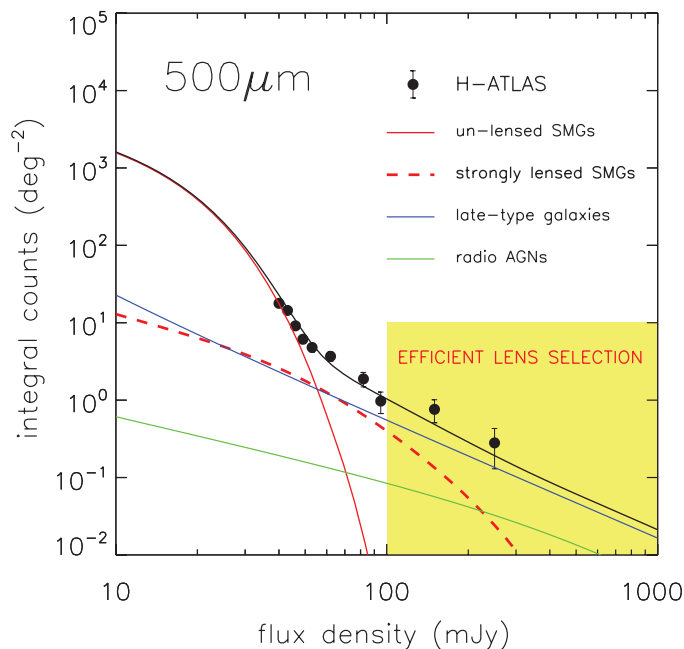


Table 1. Photometric and spectroscopic redshifts of the five lens candidates. Spectroscopic redshifts were derived from optical lines for the lens [$z_{\text{spec}}^{(\text{opt})}$] and from CO lines for the background source [$z_{\text{spec}}^{(\text{CO})}$]. Photometric redshifts are based on UV/optical/NIR photometry for the lens [$z_{\text{phot}}^{(\text{opt})}$] and H-ATLAS plus SMA and MAMBO photometry for the background source [$z_{\text{phot}}^{(\text{sub-mm/millimeter})}$], using the photometric redshift code of (36, 37)]. The quoted errors on the redshifts correspond to a 68% confidence interval (CI).

SDP ID	$z_{\text{phot}}^{(\text{opt})}$	$z_{\text{spec}}^{(\text{opt})}$	$z_{\text{phot}}^{(\text{sub-mm/millimeter})}$	$z_{\text{spec}}^{(\text{CO})}$
9	0.679 ± 0.057	—	$1.4_{-0.4}^{+0.3}$	$1.577 \pm 0.008^*$
11	0.72 ± 0.16	$0.7932 \pm 0.0012^\dagger$	$1.9_{-0.3}^{+0.4}$	$1.786 \pm 0.005^*$
17	0.77 ± 0.13	$0.9435 \pm 0.0009^\dagger$	$2.0_{-0.3}^{+0.4}$	0.942 ± 0.004 and $2.308 \pm 0.011^*$
81	0.334 ± 0.016	$0.2999 \pm 0.0002^\ddagger$	$2.9_{-0.3}^{+0.2}$	$3.037 \pm 0.010^*$ $3.042 \pm 0.0015^\parallel$ $2.6260 \pm 0.0003^\parallel$
130	0.239 ± 0.021	$0.2201 \pm 0.002^\P$	$2.6_{-0.2}^{+0.4}$	2.625 ± 0.0015 $2.6260 \pm 0.0003^\parallel$

*Datum is from CSO/Z-Spec (43).

† Datum is from the William Herschel Telescope (35).

‡ Datum is from SDSS.

§ Datum is from GB/T2pexometer (45).

$^\parallel$ Datum is from PdBI (35).

¶ Datum is from the Apache Point Observatory (35).

bright flux densities, the measured surface densities are consistent with expectations (Fig. 1) (17, 18). Exclusion of these contaminants left the five objects that form our sample of lens candidates (table S1) (35), identified as ID9, ID11, ID17, ID81, and ID130.

Unveiling the nature of the lens candidates.

For gravitational lensing systems selected at submillimeter wavelengths, we would expect the lensing galaxy to be seen in optical and/or NIR images, in which the emission from the lens dominates over the higher redshift background SMG. In line with these expectations, all of the lens candidates have a close counterpart in SDSS or UKIDSS images (or both). A likelihood ratio analysis (34) showed that the probability of a random association between these bright submillimeter sources and the close optical/NIR counterparts is less than a few percent. Therefore, the optical and submillimeter emissions must be physically related, either because they occur within the same object or because of the effects of gravitational lensing, boosting the flux of the background source and indirectly affecting the likelihood ratio calculations. The redshift measurements support the later scenario. Although the optical/NIR photometric/spectroscopic redshifts lie in the range of $z \sim 0.3$ to 0.9 (Table 1 and figs. S3 and S4) (35), the redshifts estimated from the submillimeter/millimeter spectral energy distributions (SEDs) [following the method described in (36, 37)] are distinctly different (Table 1). The lensed SMG photometric redshifts have been confirmed and made more precise through the spectroscopic detection, in these objects, of carbon monoxide (CO) rotational line emission, which are tracers of molecular gas associated to star-forming environments. Until recently, these kinds of detections were difficult to achieve without prior knowledge of the source redshift, which required extensive optical/NIR/radio follow-up observations. Because of the development of wide-bandwidth radio spectrometers capable of detecting CO lines over a wide range of redshifts, it is now possible for blind redshift measurements of SMGs to be taken without relying on optical or NIR spectroscopy (38, 39). ID81 was observed

with the Z-Spec spectrometer (40, 41) on the California Institute of Technology Submillimeter Observatory. The data revealed several CO lines redshifted into the frequency range of 187 to 310 GHz; the strongest of these lines has been interpreted as the CO J=7–6 line, with an estimated redshift of $z = 3.04$ (42). This represents the first blind redshift determination by means of Z-Spec. We followed up this observation with the PdBI and detected CO J=3–2 and CO J=5–4 emission lines, redshifted to $z = 3.042$, confirming the Z-Spec–

measured redshift (35). We also used the Zpecrometer instrument (43, 44) on the NRAO Robert C. Byrd Green Bank Telescope (GBT) to obtain an independent confirmation of the redshift of ID81 (Table 1 and fig. S1) (35, 45) and to measure the redshift of ID130. We detected redshifted CO J=1–0 emission at $z = 2.625$ in the spectrum of ID130 (fig. S1) (35, 45). This redshift was confirmed by the PdBI with the observation of CO J=3–2 and CO J=5–4 lines, yielding a redshift of $z = 2.626$ (35). The Z-Spec spectrometer

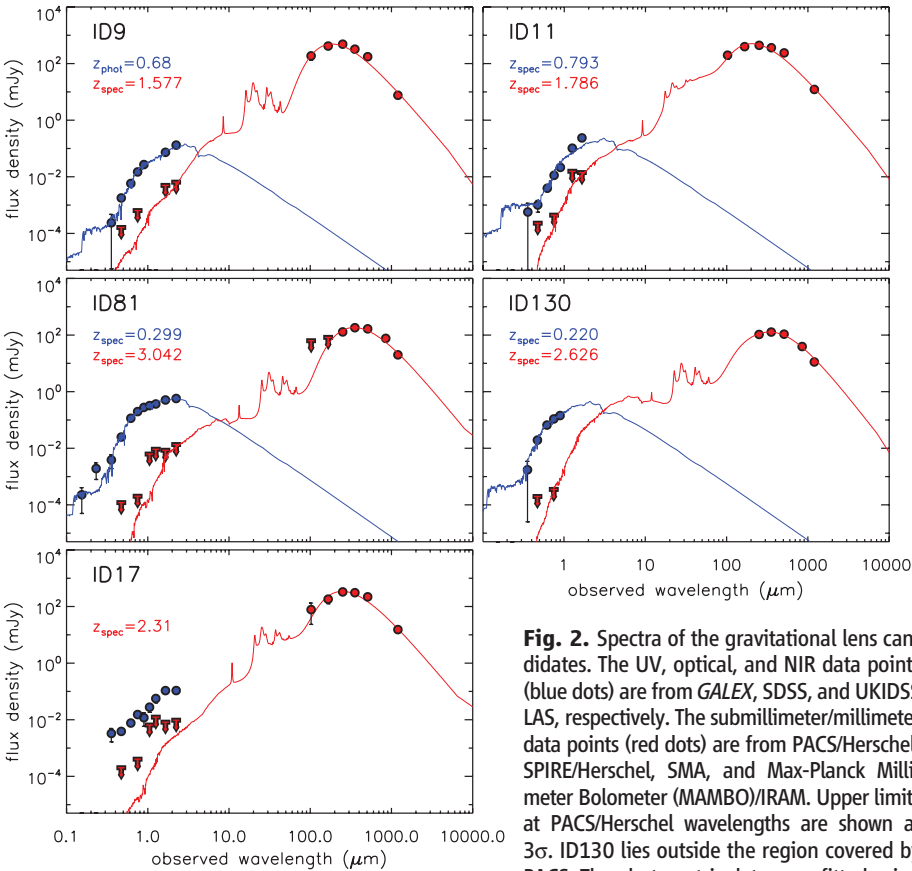


Fig. 2. Spectra of the gravitational lens candidates. The UV, optical, and NIR data points (blue dots) are from GALEX, SDSS, and UKIDSS LAS, respectively. The submillimeter/millimeter data points (red dots) are from PACS/Herschel, SPIRE/Herschel, SMA, and Max-Planck Millimeter Bolometer (MAMBO)/IRAM. Upper limits at PACS/Herschel wavelengths are shown at 3σ . ID130 lies outside the region covered by PACS. The photometric data were fitted using SED models from (47). The background source, responsible for the submillimeter emission, is a heavily dust-obscured star-forming galaxy (red solid curve), whereas the lens galaxy, which is responsible for the UV/optical and NIR part of the spectrum, is characterized by passive stellar evolution.

Table 2. Derived parameters for the five lens candidates. Estimated mass in stars (M_*) and Star Formation Rate (SFR) of the foreground galaxy derived from the best-fit to the UV/optical/NIR part of the SED; the Einstein radius measured from the SMA images (θ_E); mass within the Einstein radius (M_E) estimated from θ_E ; line-of-sight stellar velocity dispersion (σ_{FJ}^V) derived from the Faber-Jackson relation and the B-band luminosity produced by the best-fit to the UV/optical/NIR SED; Einstein radius (θ_E^{FJ}) calculated from σ_{FJ}^V ; infrared luminosity of the background source (L_{IR}), without correction for magnification, derived by fitting the submillimeter/millimeter part of

the SED and the upper limits at optical and NIR wavelengths (Fig. 2); and visual extinction parameter (A_V) inferred for the background galaxy. All the quoted errors correspond to a 68% CI. For ID17, only the infrared luminosity and the extinction parameter of the background source are quoted because the lensing mass probably consists of two galaxies that can only be disentangled in the Keck images. The symbols $M_\odot$ and $L_\odot$ denote the total mass and the total luminosity of the Sun, respectively, and correspond to $M_\odot = 1.99 \times 10^{30}$ kg and $L_\odot = 3.839 \times 10^{33}$ erg s⁻¹. Dashes indicate lack of constraint.

SDP ID	$\log(M_*) (M_\odot)$	$\log(\text{SFR}) (M_\odot \text{ yr}^{-1})$	θ_E (arc sec)	$\log(M_E) (M_\odot)$	σ_{FJ}^V (km sec ⁻¹)	θ_E^{FJ} (arc sec)	$\log(L_{IR}) (L_\odot)$	A_V
9	$10.79_{-0.11}^{+0.16}$	$-0.51_{-0.27}^{+0.20}$	—	—	232_{-56}^{+75}	$0.77_{-0.34}^{+0.49}$	$13.48_{-0.06}^{+0.07}$	$6.7_{-1.0}^{+1.5}$
11	$11.15_{-0.10}^{+0.09}$	$-0.08_{-0.24}^{+0.18}$	—	—	258_{-62}^{+82}	$0.91_{-0.40}^{+0.59}$	$13.61_{-0.06}^{+0.06}$	$5.1_{-0.7}^{+1.6}$
17	—	—	—	—	—	—	$13.57_{-0.06}^{+0.08}$	$5.3_{-0.6}^{+1.4}$
81	$11.17_{-0.08}^{+0.04}$	$-1.66_{-0.14}^{+0.46}$	1.62 ± 0.02	11.56 ± 0.01	242_{-58}^{+77}	$1.51_{-0.67}^{+0.98}$	$13.71_{-0.07}^{+0.07}$	$3.5_{-0.3}^{+3.4}$
130	$10.65_{-0.08}^{+0.06}$	$-1.17_{-0.58}^{+0.39}$	0.59 ± 0.02	10.57 ± 0.04	174_{-42}^{+55}	$0.81_{-0.36}^{+0.52}$	$13.45_{-0.09}^{+0.08}$	$1.9_{-0.3}^{+0.3}$

observed the remaining three lens candidates (42) and detected CO lines at redshifts of $z = 1.577$ and $z = 1.786$ for ID9 (fig. S2) (35) and ID11, respectively, which are higher and inconsistent with the redshifts derived from the optical photometry/spectroscopy (Table 1). The Z-Spec CO measurements for ID17 are indicative of two redshifts; one, $z = 0.942$, that is in agreement with the optical redshift and a higher one, $z = 2.31$, which is indicative of a more distant galaxy.

To determine the morphological type of the foreground galaxies, we obtained high-resolution optical images for all five objects with the Keck telescope at g - and i -bands (35). ID9, ID11, ID81, and ID130 all have optical profiles that are consistent with elliptical galaxies (figs. S5 and S6 and table S4) (35). The interpretation of the results for ID17 is complicated by the presence of two partially superimposed galaxies in the optical images (fig. S7) (35), neither exhibiting the disturbed morphology expected for lensed objects. This indicates that ID17 may be a gravitational lens system with two foreground lensing masses

at similar redshifts ($z \sim 0.8$ to 0.9)—possibly a merging system—with some molecular gas responsible for the CO emission detected by Z-Spec at $z \sim 0.9$ and confirmed with optical spectroscopy (Table 1). A fit to the UV/optical/NIR SEDs of ID9, ID11, ID81, and ID130 (46), using the models of (49), gives stellar masses in the range of 4×10^{10} to $15 \times 10^{10} M_{\odot}$ (Table 2) and almost negligible present-day star formation, which is consistent with elliptical galaxies (Fig. 2).

For all five lens systems, the background source appears to be undetected in the Keck g - and i -band images, despite the flux magnification due to lensing. After subtracting the best-fit light profile from each lens (figs. S5 to S7) (36), we found no structure that could be associated with the background source in the residual images (figs. S5 and S6). We derived $3\text{-}\sigma$ upper limits from the residual maps (table S3) and corresponding NIR limits from the UKIDSS images. These upper limits were used to fit the SEDs of the background sources assuming the models of (47), calibrated to reproduce the UV-

to-infrared SEDs of local, purely star-forming ultraluminous infrared galaxies (ULIRG) ($10^{12} \leq L_{\text{IR}}/L_{\odot} < 10^{13}$) (48). A visual extinction (49) of $A_V > 2$ is required to be consistent with the optical/NIR upper limits (Fig. 2 and Table 2), confirming severe dust obscuration in these galaxies along the line of sight. Our results indicate that these submillimeter bright gravitationally lensed galaxies would have been entirely missed by standard optical methods of selection.

We obtained observations at the SMA for ID81 and ID130 at $880 \mu\text{m}$, with the aim of detecting the lensed morphology of the background galaxy (35). The SMA images reveal extended submillimeter emission distributed around the cores of the foreground elliptical galaxies, with multiple peaks (four main peaks in ID81 and two in ID130), which is consistent with a lensing interpretation of these structures (Fig. 3). The position of these peaks can be used to directly constrain the Einstein radius—the radius of the circular region on the sky (the Einstein ring) into which an extended source would be lensed if a foreground galaxy were exactly along the line of sight of the observer to the source (for a perfectly circular lens). The Einstein radius is a measure of the projected mass of the lens, so it can be used to derive the total (dark plus luminous) mass of the galaxy within the Einstein radius (Table 2) (35). Another measure of the total mass of a lens is the line-of-sight stellar velocity dispersion, σ_v . We have estimated σ_v from the local Faber–Jackson (FJ) relation (50) between σ_v and the rest-frame B-band luminosity for elliptical galaxies. Assuming passive stellar evolution for the lens galaxies, which is appropriate for elliptical galaxies, we have extrapolated their rest-frame K-band luminosity to $z = 0$ [using the evolutionary tracks of

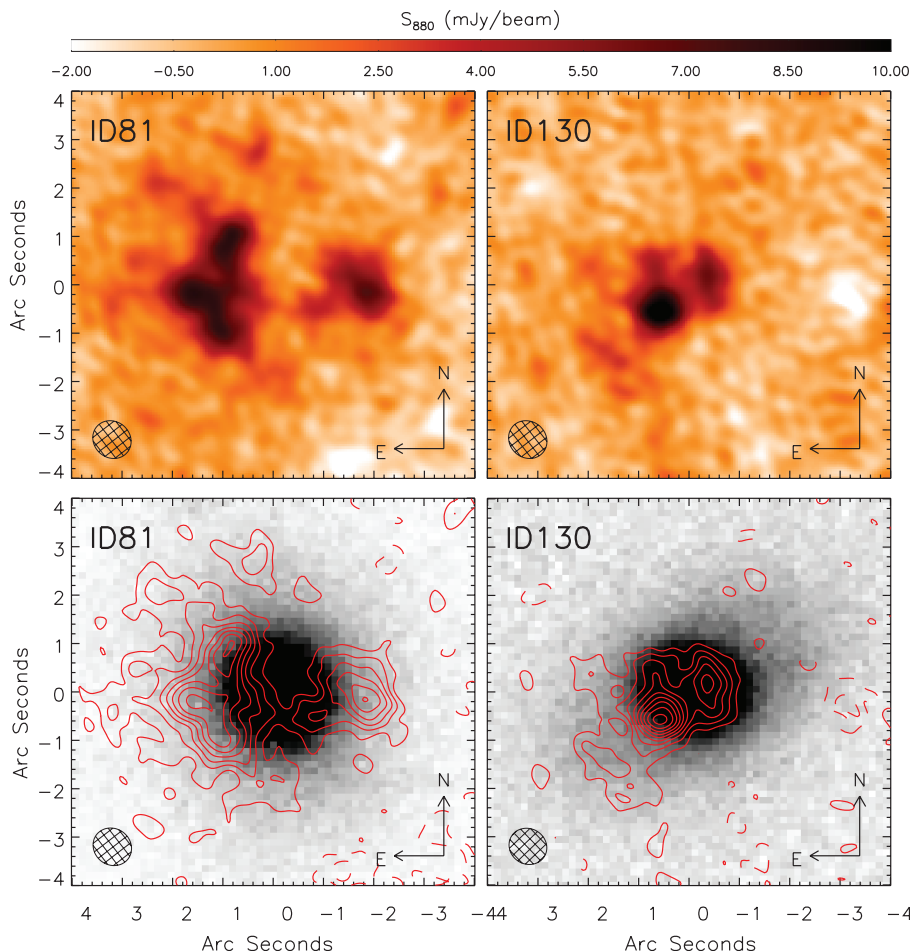


Fig. 3. Submillimeter and optical follow-up imaging of ID81 and ID130. The SMA images of ID81 and ID130 are shown in the top panels, centered on the optical counterpart, and were obtained by combining the visibility data from very extended, compact, and subcompact configuration observations. The Keck i -band image of ID81 and ID130 are shown in the bottom panels with the SMA contours superimposed (in red). The contours are in steps of -2σ , 2σ , 4σ , 6σ , 8σ , 10σ ..., with $\sigma = 0.6 \text{ mJy/beam}$. The SMA synthesized beam is shown in the bottom-left corner.

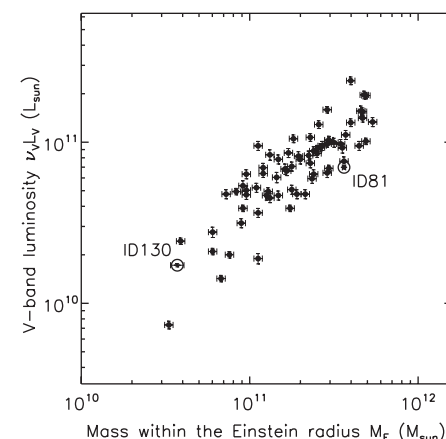


Fig. 4. Relationship between mass and luminosity for the lensing galaxy in ID81 and ID130. The rest-frame V -band luminosity was derived from the best-fit SED to the UV/optical/NIR photometric data; the mass within the Einstein radius is that measured directly from the SMA images. The light versus mass relation inferred for ID81 and ID130 (open circles) is consistent with that observed for the SLACS lenses [black dots, from (54), assuming an uncertainty of 0.025 dex in their mass estimates].

(51)] and then converted this to B-band luminosity using the $B - K = 4.43$ color relation from (52). The result was then applied to the FJ relation from (53). Given a mass model for the lens (35), we can predict the Einstein radius of the galaxy from the value of σ_v expected from the FJ relation and compare it with that directly measured from the SMA images (Table 2). Although the value of the Einstein radius derived from the line-of-sight stellar velocity dispersion is affected by large uncertainties (as a result of the scatter in the FJ relation), it is consistent with the value measured in the SMA images for both ID81 and ID130. In order to test whether the properties of the lensing galaxies in our sample are consistent with those of other known lens ellipticals at similar redshift, we compared the V-band mass-to-light ratio of the lens galaxy for ID81 and ID130 with those measured in the Sloan Lens Advanced Camera for Surveys (ACS) Survey (SLACS) (Fig. 4) (54), which cover a similar redshift range ($z \sim 0.1$ to 0.3). The agreement with the average trend revealed by SLACS confirms that our lens selection method is not biased to lensing ellipticals with atypical luminosities. Moreover, the location of ID130 in Fig. 4 indicates that our selection method can probe lower masses and lower luminosity lens galaxies than those sampled by SLACS, thus offering a wider range in lens properties to be investigated.

The best-fit SED to the submillimeter/millimeter photometry for each of the five background sources give infrared luminosities $L_{\text{IR}} \geq \sim 3 \times 10^{13} L_{\odot}$ (Table 2), which would classify these objects as Hyper Luminous Infra-Red galaxies (HLIRGs) ($L_{\text{IR}} \geq 10^{13} L_{\odot}$). However, a correction for magnification because of lensing will reduce these values by a factor of 10 or greater. For example, assuming that the light distribution of the background source is described by a Gaussian profile with a full width at half maximum (FWHM) of 0.2 arcseconds [which is consistent with the physical extension of the background galaxy in (4)], the best-fit lens model (fig. S9) (35) predicts total amplifications of ~ 19 and ~ 6 for ID81 and ID130, respectively. Typical amplifications of 8 to 10 are also suggested by (14); therefore, it is more likely that these sources are ULIRGs.

These results already provide constraints for models of the formation and evolution of massive galaxies at high redshift. The fact that many (if not all) of the brightest SMGs detected in the H-ATLAS SDP field are amplified by lensing implies that unlensed $z > 1$ star-forming galaxies with flux densities of more than 100 mJy at 500 μm are rare, with ≤ 4.6 of them per 14.4 deg^{-2} , at 99% probability (assuming Poisson statistics). This translates into a 0.32 deg^{-2} upper limit on the surface density of these sources. The same limit should translate to the abundance of HLIRGs with $L_{\text{IR}} > 5 \times 10^{13} L_{\odot}$ at $z < 4$ because they would also have 500- μm flux densities above 100 mJy, which has possible implications for the role of feedback during the formation of the most massive galaxies in the universe. By extrapolat-

ing our SDP findings to the full H-ATLAS field, we predict a total sample of more than 100 bright-lensed sources, with which we can further improve this constraint.

References and Notes

- When multiple images of the same background source are formed, the event is known as strong gravitational lensing.
- P. J. Marshall *et al.*, *Astrophys. J.* **671**, 1196 (2007).
- D. P. Stark *et al.*, *Nature* **455**, 775 (2008).
- A. M. Swinbank *et al.*, *Nature* **464**, 733 (2010).
- I. Smail, R. J. Ivison, A. W. Blain, *Astrophys. J.* **490**, L5 (1997).
- A. W. Blain, J. Kneib, R. J. Ivison, I. Smail, *Astrophys. J.* **512**, L87 (1999).
- M. Rowan-Robinson *et al.*, *Nature* **351**, 719 (1991).
- I. Smail, R. J. Ivison, A. W. Blain, J. Kneib, *Mon. Not. R. Astron. Soc.* **331**, 495 (2002).
- J. D. Vieira *et al.*, *Astrophys. J.* **719**, 763 (2010).
- G. L. Pilbratt *et al.*, *Astron. Astrophys.* **518**, L1 (2010) (10.1051/0004-6361/201014759).
- A. W. Blain, *Mon. Not. R. Astron. Soc.* **283**, 1340 (1996).
- F. Perrotta, C. Baccigalupi, M. Bartelmann, G. De Zotti, G. L. Granato, *Mon. Not. R. Astron. Soc.* **329**, 445 (2002).
- F. Perrotta *et al.*, *Mon. Not. R. Astron. Soc.* **338**, 623 (2003).
- M. Negrello *et al.*, *Mon. Not. R. Astron. Soc.* **377**, 1557 (2007).
- K. Coppin *et al.*, *Mon. Not. R. Astron. Soc.* **372**, 1621 (2006).
- S. C. Chapman, A. W. Blain, I. Smail, R. J. Ivison, *Astrophys. J.* **622**, 772 (2005).
- S. Serjeant, D. Harrison, *Mon. Not. R. Astron. Soc.* **356**, 192 (2005).
- G. de Zotti *et al.*, *Astron. Astrophys.* **431**, 893 (2005).
- L. V. E. Koopmans, T. Treu, A. S. Bolton, S. Burles, L. A. Moustakas, *Astrophys. J.* **649**, 599 (2006).
- I. W. A. Browne, The CLASS Collaboration, *Astron. Soc. Pac. Conf. Ser.* **237**, 15 (2001).
- M. Oguri *et al.*, *Astron. J.* **132**, 999 (2006).
- C. Faure *et al.*, *Astrophys. J.* **176**, 19 (2008).
- A. Weiß *et al.*, *Astrophys. J.* **707**, 1201 (2009).
- M. J. Devlin *et al.*, *Nature* **458**, 737 (2009).
- S. Eales *et al.*, *Publ. Astron. Soc. Pac.* **122**, 499 (2010).
- M. J. Griffin *et al.*, *Astron. Astrophys.* **518**, L3 (2010).
- A. Poglitsch *et al.*, *Astron. Astrophys.* **518**, L2 (2010).
- E. Ibar *et al.*, *Astrophys. J.*, in press; preprint available at <http://xxx.lanl.gov/abs/astro-ph/1009.0262>.
- S. Driver *et al.*, *Astrophys. J.*, in press; preprint available at <http://xxx.lanl.gov/abs/astro-ph/1009.0614>.
- S. J. Maddox *et al.*, *Astron. Astrophys.* **518**, L11 (2010).
- D. L. Clements *et al.*, *Astron. Astrophys.* **518**, L8 (2010).
- M. Baes *et al.*, *Astron. Astrophys.* **518**, L39 (2010).
- J. González-Nuevo *et al.*, *Astron. Astrophys.* **518**, L38 (2010).
- D. J. B. Smith *et al.*, *MNRAS*, in press; preprint available at <http://arxiv.org/abs/1007.5260>.
- Supporting online material (SOM) text is available on Science Online.
- D. H. Hughes *et al.*, *Mon. Not. R. Astron. Soc.* **335**, 871 (2002).
- I. Aretxaga *et al.*, *Mon. Not. R. Astron. Soc.* **342**, 759 (2003).
- A. Weiß *et al.*, *Astrophys. J.* **705**, L45 (2009).
- E. Daddi *et al.*, *Astrophys. J.* **695**, L176 (2009).
- B. J. Naylor *et al.*, *Soc. Photo-Opt. Instrum. Eng. (SPIE) Conf. Ser.* **4855**, 239 (2003).
- C. M. Bradford *et al.*, *Astrophys. J.* **705**, 112 (2009).
- R. Lupu, submitted (available at <http://xxx.lanl.gov/abs/astro-ph/1009.5983>) (2010).
- A. I. Harris *et al.*, *Astron. Soc. Pac. Conf. Ser.* **375**, 82 (2007).
- The Zpectrometer frequency coverage allows it to search for CO J=1–0 transition over the redshift range of 2.2 to 3.5 simultaneously.
- D. Frayer *et al.*, *Astrophys. J.*, in press; preprint available at <http://xxx.lanl.gov/abs/astro-ph/1009.2194>.
- Only g- and i-band photometry can be obtained for each of the two galaxies detected in ID17. For this reason, we did not attempt any fit to the optical/NIR SED of this H-ATLAS source.
- E. da Cunha, S. Charlot, D. Elbaz, *Mon. Not. R. Astron. Soc.* **388**, 1595 (2008).
- E. da Cunha *et al.*, *Astrophys. J.*, in press; preprint available at <http://xxx.lanl.gov/abs/astro-ph/1008.2000>.
- The visual extinction parameter, A_v , is the difference between the observed V-band magnitude and the intrinsic (dust-free) V-band magnitude of the galaxy.
- S. M. Faber, R. E. Jackson, *Astrophys. J.* **204**, 668 (1976).
- C. J. Willott, S. Rawlings, M. J. Jarvis, K. M. Blundell, *Mon. Not. R. Astron. Soc.* **339**, 173 (2003).
- J. Huang, L. L. Cowie, G. A. Luppino, *Astrophys. J.* **496**, 31 (1998).
- D. H. Gudehus, *Astrophys. J.* **382**, 1 (1991).
- M. W. Auger *et al.*, *Astrophys. J.* **705**, 1099 (2009).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6005/800/DC1
SOM Text
Figs. S1 to S9
Tables S1 to S4
References

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The Effective Fine-Structure Constant of Freestanding Graphene Measured in Graphite

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Electrons in graphene behave like Dirac fermions, permitting phenomena from high-energy physics to be studied in a solid-state setting. A key question is whether or not these fermions are critically influenced by Coulomb correlations. We performed inelastic x-ray scattering experiments on crystals of graphite and applied reconstruction algorithms to image the dynamical screening of charge in a freestanding graphene sheet. We found that the polarizability of the Dirac fermions is amplified by excitonic effects, improving screening of interactions between quasiparticles. The strength of interactions is characterized by a scale-dependent, effective fine-structure constant, $\alpha_g^*(\mathbf{k}, \omega)$, the value of which approaches $0.14 \pm 0.092 \sim 1/7$ at low energy and large distances. This value is substantially smaller than the nominal $\alpha_g = 2.2$, suggesting that, on the whole, graphene is more weakly interacting than previously believed.

Graphene is a single layer of carbon atoms with an unusual electronic structure that mimics the massless Dirac equation, allowing phenomena familiar from high-energy physics to be investigated in a solid-state setting (1). Because of its low density of states near the Fermi level, it is possible to tune the effective carrier density of graphene with a gate voltage. This makes graphene the potential foundation for a new generation of low-cost, flexible electronics (1).

It is widely believed that graphene, if isolated from substrate effects, should be a strongly interacting electron system. (1–8) The strength of Coulomb interactions in graphene is measured by the ratio of its potential energy to its kinetic energy, $U/K = e^2/\hbar v_F = 2.2$, where e is the charge of an electron, $\hbar$ is Planck's constant, and v_F is the Fermi velocity of the Dirac particles. This ratio is independent of the carrier density and is usually referred to as the fine-structure constant, α_g . Unlike the analogous quantity $\alpha = 1/137$ in quantum electrodynamics (QED), α_g is greater than unity; thus, there is no small expansion parameter for electromagnetic interactions, which have been predicted to lead to novel ground states such as an excitonic insulator (3) or a perfect fluid that might exhibit electronic turbulence (4).

Surprisingly, so far there is little direct evidence for strong interactions in graphene. The hallmark of interactions is a logarithmically divergent renormalization of the Fermi velocity, v_F (5, 8). However, this effect has not been observed in either angle-resolved photoemission spectroscopy

(ARPES) experiments (9, 10) or in scanning single-electron transistor (SET) measurements of the electronic compressibility (11). A recent optical infrared measurement observed a departure from the noninteracting spectrum (12); however, the effect is not logarithmic and does not agree with ARPES or SET measurements. Interaction effects have been observed in high magnetic fields, but in this case the kinetic energy is quenched by the formation of Landau levels (13–15). Some of these measurements were done on supported graphene, which can suppress interactions through substrate dielectric screening. However, recent measurements show that free-

standing graphene in zero field also behaves like a simple semimetal (16).

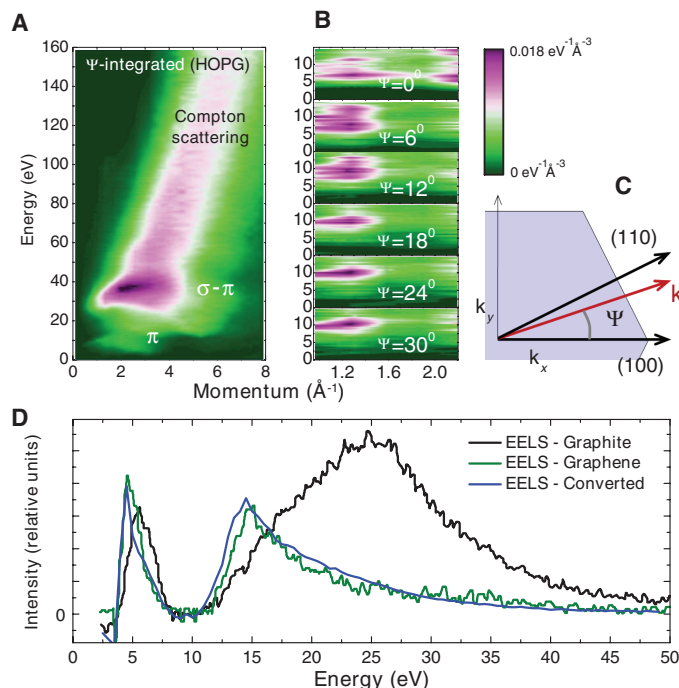
The absence of a v_F renormalization seems irreconcilable with a large value of the fine-structure constant. However, the particles measured in experiments are not bare electrons, but dressed quasiparticles, which interact via the screened Coulomb interaction (17). Hence, a better measure of the strength of interactions is the dressed fine-structure constant, $\alpha_g^*(\mathbf{k}, \omega) = \alpha_g/\epsilon(\mathbf{k}, \omega) = \alpha_g[1 + V(k)\chi(\mathbf{k}, \omega)]$, where $V(k) = 2\pi e^2/k$ is the bare Coulomb interaction in two dimensions, $\epsilon(\mathbf{k}, \omega)$ is the dielectric function, and $\chi(\mathbf{k}, \omega)$ is the charge response function of graphene. Unlike α_g , $\alpha_g^*(\mathbf{k}, \omega)$ describes the retarded interaction among the dressed quasiparticles and accounts for the influence of screening generated dynamically within the Dirac system (18). $\alpha_g^*(\mathbf{k}, \omega)$ is not a “background” dielectric constant, but a parameter that accounts for the dynamically generated screening by the valence electrons. Diagrammatic calculations may be structured in powers of $\alpha_g^*(\mathbf{k}, \omega)$, so this function can be considered a valid expansion parameter (19).

To determine $\alpha_g^*(\mathbf{k}, \omega)$, one must determine the response function, $\chi(\mathbf{k}, \omega)$, which is a general representation of the charge dynamics of the system. In real space, $\chi(\mathbf{r}_1 - \mathbf{r}_2, t)$ represents the amplitude that a disturbance in the electron density at $\mathbf{r}_1$ will propagate to $\mathbf{r}_2$ after an elapsed time, t . $\chi(\mathbf{k}, \omega)$ also describes, in linear response theory, how the system responds to charged perturbations via

$$n_{ind}(\mathbf{k}, \omega) = V(\mathbf{k}) \chi(\mathbf{k}, \omega) n_{ext}(\mathbf{k}, \omega) \quad (1)$$

where $n_{ext}(\mathbf{k}, \omega)$ is an arbitrary source and $n_{ind}(\mathbf{k}, \omega)$ is the charge induced in the medium (20).

Fig. 1. IXS experiments from graphite and extraction of the response function for graphene. (A) Scattered intensity as a function of energy and momentum for highly oriented pyrolytic graphite (HOPG), which gives the Ψ -integrated response. (B) Angle-resolved spectra for the domain over which anisotropy was observed. (C) Brillouin zone of graphene with various vectors defined. (D) Test of Eq. 4 on the electron energy loss experiments of Eberlein (24) (fit value $k = 0.33 \text{ \AA}^{-1}$), showing that an accurate response for graphene can be obtained from IXS experiments on graphite.



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To determine $\chi(\mathbf{k}, \omega)$ for graphene, we performed inelastic x-ray scattering (IXS) experiments on single crystals of graphite, which consists of layers of graphene loosely bound by electrostatic van der Waals forces. IXS measures the dynamic structure factor, $S(\mathbf{k}, \omega)$, which is related to the imaginary part of the charge response through the quantum mechanical version of the fluctuation-dissipation theorem (20, 21)

$$S(\mathbf{k}, \omega) = -\frac{1}{\pi} \frac{1}{1 - e^{-\hbar\omega/kT}} \text{Im}[\chi_{3D}(\mathbf{k}, -\mathbf{k}, \omega)] \quad (2)$$

where T is the temperature and χ_{3D} is the charge response of graphite. Equation 2 provides only the imaginary part of χ_{3D} ; if a sufficiently broad energy spectrum is sampled in the experiment, the real part may be determined by the Kramers-Kronig (KK) transform (21, 19). We note that, because graphite is layered, its electron density is nonuniform, so $\chi_{3D} = \chi_{3D}(\mathbf{k}, \mathbf{G} - \mathbf{k}, \omega)$ is a function of two momenta, $\mathbf{G}$ being a reciprocal lattice vector. IXS experiments provide the $\mathbf{G} = 0$ component (21, 19).

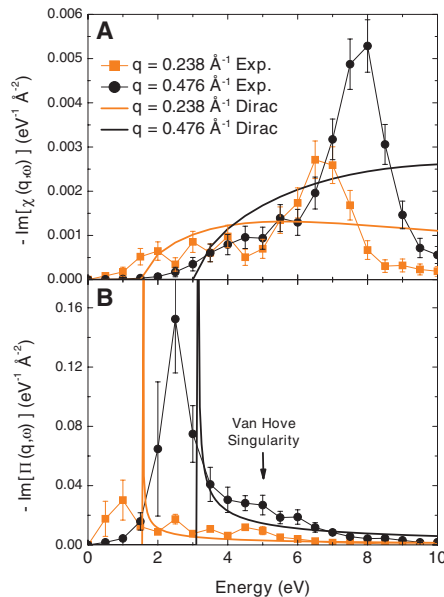


Fig. 2. Response functions for graphene in the low-momentum region, showing the Dirac particles. **(A)** $-\text{Im}[\chi(\mathbf{k}, \omega)]$. The Dirac spectrum is visible as a continuum below the π plasmon, which arises from the Van Hove singularity at the top of the π band. Also shown is the expected response for idealized, noninteracting Dirac fermions, using Eq. 3 and $\Pi(\mathbf{k}, \omega) = -k^2/(4\sqrt{(\hbar v_F q)^2 - \omega^2})$, with $v_F = 10^6$ m/s. The curves are plotted on an absolute scale, and the magnitudes may be directly compared. **(B)** Experimental and idealized $-\text{Im}[\Pi(\mathbf{k}, \omega)]$, showing the locations of single-particle excitations. The peak in the measured response is 0.6 eV lower in energy than the ideal response, indicating excitonic effects, which transfer spectral weight from the Van Hove singularity to the Dirac spectrum.

The IXS spectra, shown in Fig. 1, A and B (19), are dominated by two collective excitations, at approximately 6 eV and 35 eV, which were observed in previous studies (22, 23) and are normally referred to as the π and σ - π plasmons, respectively. These excitations are, however, not plasmons in the usual sense; they do not arise from free carrier screening but from Van Hove singularities in the band structure. The former resides at the edge of the π bands near the M point in the Brillouin zone, and the latter in the σ -bonded bands along the A-L line (23). Beneath the plasmons, a continuum of single-particle excitations is visible. The spectra were measured, for $k_z = 0$, throughout an entire, symmetry-independent sector of the Brillouin zone (Fig. 1C).

Although these experiments were done on graphite crystals, the results are directly relevant to graphene. Graphite is a quasi-two-dimensional system in which the interlayer hopping, $t_\perp = 0.4$ eV, is much smaller than that in-plane, $t_\parallel = 3$ eV. At

energy scales greater than $t_\perp$, the π band of graphite is essentially the same as in graphene, and the system can be thought of as a stack of graphene sheets coupled only by direct, long-ranged Coulomb interactions.

More quantitatively, graphite and graphene have approximately the same polarizability, $\Pi(\mathbf{k}, \omega)$. Physically, Π can be thought of as the response of the system, excluding direct Coulomb interactions, which couple the layers, and is related to the response function by

$$\chi(\mathbf{k}, \omega) = \frac{\Pi(\mathbf{k}, \omega)}{1 - V(\mathbf{k})\Pi(\mathbf{k}, \omega)} \quad (3)$$

Making the assumption that Π is the same for both graphite and graphene, and that the graphene sheets are very thin, we acquire an expression (19) for the response of graphene in terms of that for graphite

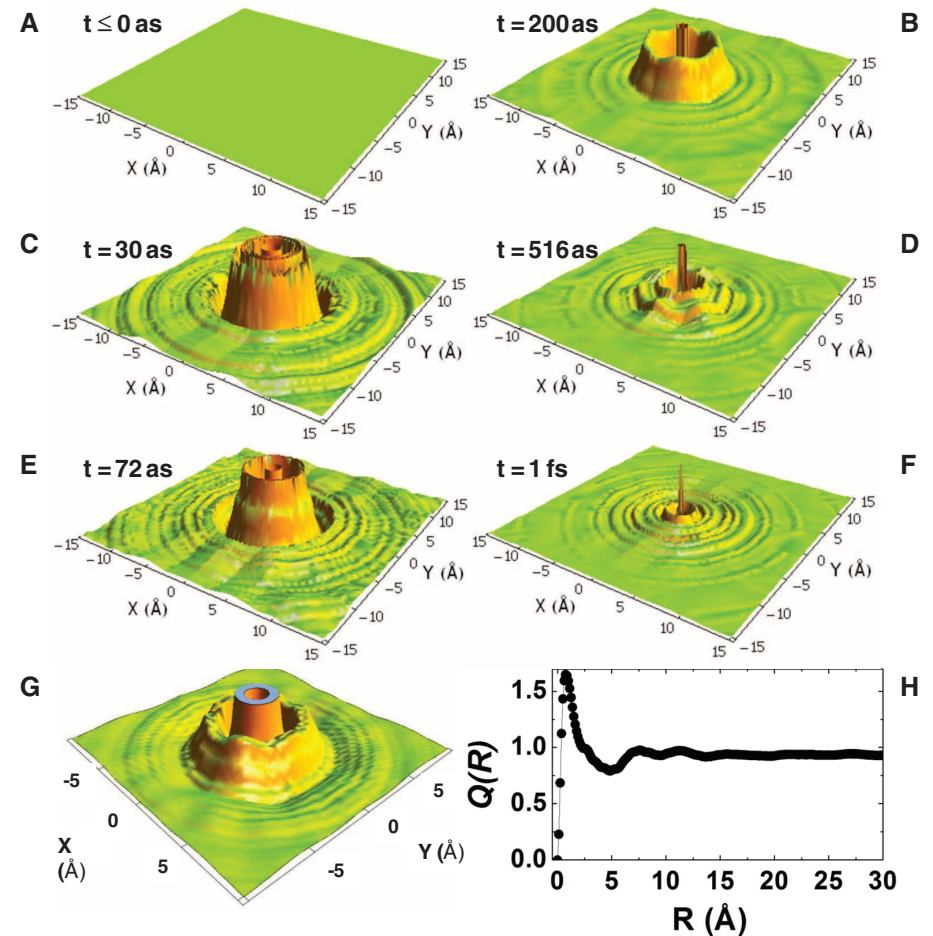


Fig. 3. Dynamics of the valence electrons in graphene. **(A to F)** Dynamical electron density, $n_{ind}(x, y, t)$, induced by a point source appearing at the origin and then instantaneously disappearing. We have taken $e = 1$. Densities are plotted over a field of view of $30 \text{ \AA}$ by $30 \text{ \AA}$ at selected times. The vertical scale has been truncated at $\pm 5.0 \text{ \AA}^{-2}$ to magnify the finer features. The resolutions for these images were $\Delta r = 0.20 \text{ \AA}$ and $\Delta t = 10.3$ as. The time resolution is fast in comparison with currently available laser pulses. **(G)** Time-independent electron density, $n_{ind}(x, y)$, induced by a static point charge. The vertical scale has been truncated at $\pm 0.04 \text{ \AA}^{-2}$ to magnify the finer features. **(H)** Integrated density $Q(R)$, giving an asymptotic value of $Q(\infty) = (0.924 \pm 0.046)e$, showing that a static point charge in graphene is screened nearly completely.

$$\chi(\mathbf{k}, \omega) = \frac{\chi_{3D}(\mathbf{k}, -\mathbf{k}, \omega) \cdot d}{1 - V(\mathbf{k})[1 - F(\mathbf{k})]\chi_{3D}(\mathbf{k}, -\mathbf{k}, \omega) \cdot d} \quad (4)$$

where $d = 3.35 \text{ \AA}$ is the distance between the layers and

$$F(\mathbf{k}) = \frac{\sinh(qd)}{\cosh(qd) - \cos(k_z d)} \quad (5)$$

is a form factor that describes the layered structure of graphite, q and k_z being the magnitude of the in- and out-of-plane components of $\mathbf{k}$, respectively (19). Equation 4 can be thought of as a means of turning off the Coulomb interaction between the layers, revealing the response for half-filled, freestanding graphene.

To test Eq. 4, we applied it to an electron energy loss (EELS) study by Eberlein (24) of the dielectric loss function $-\text{Im}[1/\epsilon(\mathbf{k}, \omega)]$, which is proportional to $\text{Im}[\chi]$, for both graphite and freestanding graphene. They found that the π and σ - π plasmons are also present in graphene but are shifted to lower energy compared with graphite because of the reduced dimensionality (23, 24). We scaled their graphite spectra with the f-sum rule, KK transformed, applied our Eq. 4, and compared the results to their spectra for

graphene (Fig. 1D). The curves match nearly exactly, reproducing the red shifts and changes in spectral weight of the two plasmons. We conclude that Eq. 4 provides a valid response function for freestanding graphene at energy scales greater than $t_\perp$ (19).

At the lowest momenta measured, the $\chi(\mathbf{k}, \omega)$ derived from Eq. 4 shows signatures of the Dirac fermions. In Fig. 2A, we plot $-\text{Im}[\chi(\mathbf{k}, \omega)]$ as a function of ω for two momenta at which the quantity $\hbar v_F k$ is less than the energy of the Van Hove singularity in the π band. Also shown is the spectrum for idealized, noninteracting Dirac fermions. A continuum is visible below 5 eV whose magnitude and dispersion with $\mathbf{k}$ are close to that expected for Dirac particles. This suggests that the low-frequency, long-wavelength response of graphene is strongly influenced by the Dirac fermions. The experimental and idealized spectra differ, however, in two respects. First, the curves deviate near the energy of the π plasmon, because in the real material the width of the π band is finite. Second, the experimental onset energy is lower and smoother than expected.

The origin of these discrepancies is clarified by examining the polarization function, $-\text{Im}[\Pi(\mathbf{k}, \omega)]$, shown in Fig. 2B, which exhibits peaks at the energy of single-particle transitions rather than of the collective modes. Again, the curves for idealized Dirac fermions are shown. The peak in the data is broader and shifted to lower energy, by approximately 0.6 eV, compared with the idealized spectrum. Further, the spectral weight in the Van Hove singularity is suppressed from what is expected (23), being reduced to a shoulder on the Dirac spectrum. These effects may be understood as arising from a combination of band curvature (i.e., deviation from the Dirac spectrum at high energy), as well as excitonic effects, which are known to transfer spectral weight to lower energy in particle-hole spectra. We note that the 0.6-eV shift is similar in magnitude to that predicted by recent ab initio calculations employing the Bethe-Salpeter equation (25, 26).

To observe how excitations in graphene propagate in real time, in Fig. 3, A to F, we plot the time-dependent charge density, $n_{ind}(\mathbf{r}, t)$, arising from a point source in graphene, determined by using $n_{ext}(\mathbf{k}, \omega) = -e$ in Eq. 1. As expected from causality, $n_{ind} = 0$ for $t < 0$. The earliest response ($t < 200$ as) is dominated by the fast, broadband, σ - π plasmon, which is isotropic and $\sim 5 \text{ \AA}$ in size because of the localized character of the sp^2 bonds. The narrower π plasmon emerges later ($t > 200$ as), extends over $\sim 10 \text{ \AA}$, and exhibits six-fold rotational symmetry. One can think of the sequence of events as a localized burst of density that back-scatters off the atomic lattice.

The effective fine-structure constant, $\alpha_g^*(\mathbf{k}, \omega)$, is shown in Fig. 4. $\alpha_g^*(\mathbf{k}, \omega)$ is a valid coupling constant at low energy where the bands are Dirac-like; at high energy, it may be thought of simply as the inverse dielectric function, scaled

by α_g . Figure 4 shows that α_g^* is complex, and is not a constant but is a strong function of frequency and momentum. This indicates that the strength of interactions in graphene depends on the scale on which the system is probed. The magnitude of α_g^* ranges from greater than 2 at high energy to much less than unity at energies lying below the $\omega = \hbar v_F k$ line (Fig. 4).

An important limit is that of zero frequency and small momentum. At the lowest momenta measured, we found that $\Pi(\mathbf{k}, 0) \sim k$ as $k \rightarrow 0$ (19). Extrapolating linearly to zero, we find that $\alpha_g^*(0^+, 0) \equiv \lim_{k \rightarrow 0} \alpha_g^*(\mathbf{k}, 0) = 0.14 \pm 0.092 \approx$

$1/7$, which may be thought of as a static dielectric constant of $\epsilon = [1 - Q(\infty)/e]^{-1} = 15.4^{+39.56}_{-6.45}$ (19). This large value, which is an outcome of the excitonic shifts shown in Fig. 2B, is 3.5 times as large as past estimates based on the random phase approximation (RPA) (27) or GW methods (26) in which excitonic effects were neglected. The small value of α_g^* in this limit indicates that graphene can screen very effectively over finite distances and should act like a weakly interacting system for phenomena that take place at low energy and modest wave vector.

For illustration, we simulate the response of the system to a charged impurity by solving Eq. 1 with $n_{ext}(\mathbf{k}, 0) = -e$. The induced charge $n_{ind}(\mathbf{r})$ (Fig. 3G) is a cloud $\sim 10 \text{ \AA}$ in size, comprising an isotropic inner layer surrounded by a six-fold symmetric outer layer, reminiscent of the excitations in Fig. 3, A to F. Figure 3H shows $Q(R)$, which is the total charge contained in $n_{ind}(\mathbf{r})$ within a shell of radius R . $Q(R)$ oscillates and plateaus at large R to the value $Q(\infty) = (0.924 \pm 0.046)e$. Hence, at length scales larger than a few nanometers, a charged impurity in graphene is screened nearly completely. This explains the surprisingly small sensitivity of the mobility of graphene to a high- κ dielectric environment (28, 29), which was anticipated to reduce scattering from charged impurities. Because graphene already screens impurities very efficiently, immersing it in a high- κ environment does not substantially influence its properties. Screening effects may also explain the absence of a detectable velocity renormalization in recent ARPES and SET experiments. Overall, our results indicate that graphene is a more weakly interacting system than previously assumed and that the mobility of graphene may currently be limited by some phenomenon other than scattering from charged impurities.

References and Notes

1. A. H. Castro Neto, F. Guinea, N. M. R. Peres, K. S. Novoselov, A. K. Geim, *Rev. Mod. Phys.* **81**, 109 (2009).
2. K. S. Novoselov *et al.*, *Science* **306**, 666 (2004).
3. J. E. Drut, T. A. Lähde, *Phys. Rev. Lett.* **102**, 026802 (2009).
4. M. Müller, J. Schmalian, L. Fritz, *Phys. Rev. Lett.* **103**, 025301 (2009).
5. J. González, F. Guinea, M. A. H. Vozmediano, *Nucl. Phys. B* **424**, 595 (1994).
6. D. E. Sheehy, J. Schmalian, *Phys. Rev. Lett.* **99**, 226803 (2007).

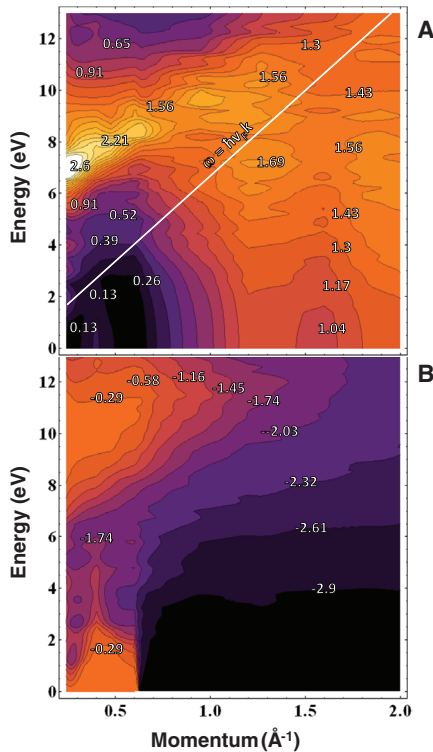


Fig. 4. The effective, screened fine-structure constant, $\alpha_g^*(\mathbf{k}, \omega)$, as defined in the text. (A) The magnitude of $\alpha_g^*(\mathbf{k}, \omega)$, plotted against momentum and energy. The Dirac dispersion $\omega = \hbar v_F k$ is indicated by the white line. In the low momentum region, $\alpha_g^*(\mathbf{k}, \omega)$ is larger above this line than below. (B) The phase of $\alpha_g^*(\mathbf{k}, \omega)$, in radians.

7. Y. Barlas, T. Pereg-Barnea, M. Polini, R. Asgari, A. H. MacDonald, *Phys. Rev. Lett.* **98**, 236601 (2007).
8. E. H. Hwang, B. Y.-K. Hu, S. Das Sarma, *Phys. Rev. Lett.* **99**, 226801 (2007).
9. S. Y. Zhou *et al.*, *Nat. Phys.* **2**, 595 (2006).
10. A. Bostwick, T. Ohta, T. Seyller, K. Horn, E. Rotenberg, *Nat. Phys.* **3**, 36 (2007).
11. J. Martin *et al.*, *Nat. Phys.* **4**, 144 (2008).
12. Z. Q. Li *et al.*, *Nat. Phys.* **4**, 532 (2008).
13. K. I. Bolotin, F. Ghahari, M. D. Shulman, H. L. Stormer, P. Kim, *Nature* **462**, 196 (2009).
14. X. Du, I. Skachko, F. Duerr, A. Luican, E. Y. Andrei, *Nature* **462**, 192 (2009).
15. J. C. Checkelsky, L. Li, N. P. Ong, *Phys. Rev. B* **79**, 115434 (2009).
16. X. Du, I. Skachko, A. Barker, E. Y. Andrei, *Nat. Nanotechnol.* **3**, 491 (2008).
17. D. Pines, P. Nozières, *The Theory of Quantum Liquids* (Perseus Books, Cambridge, MA, 1999).
18. F. Aryasetiawan, O. Gunnarsson, *Rep. Prog. Phys.* **61**, 237 (1998).
19. Materials and methods are available as supporting material on Science Online.
20. W. Schülke, *Electron Dynamics by Inelastic X-Ray Scattering* (Oxford Univ. Press, Oxford, 2007).
21. P. Abbamonte *et al.*, *Adv. Mater.* **22**, 1141 (2010).
22. N. Hiraoka, H. Ishii, I. Jarrige, Y. Q. Cai, *Phys. Rev. B* **72**, 75103 (2005).
23. A. G. Marinopoulos, L. Reining, A. Rubio, V. Olevano, *Phys. Rev. B* **69**, 245419 (2004).
24. T. Eberlein *et al.*, *Phys. Rev. B* **77**, 233406 (2008).
25. L. Yang, J. Deslippe, C.-H. Park, M. L. Cohen, S. G. Louie, *Phys. Rev. Lett.* **103**, 186802 (2009).
26. P. E. Trevisanuto, M. Holzmann, M. Côté, V. Olevano, *Phys. Rev. B* **81**, 121405 (2010).
27. M. Polini, A. Tomadin, R. Asgari, A. H. MacDonald, *Phys. Rev. B* **78**, 115426 (2008).
28. L. A. Ponomarenko *et al.*, *Phys. Rev. Lett.* **102**, 206603 (2009).
29. C. Jang *et al.*, *Phys. Rev. Lett.* **101**, 146805 (2008).
30. We gratefully acknowledge helpful discussions with A. H. MacDonald, D. Maslov, P. Guinea, L. Levitov, and A. J. Millis, and Y. Cai for supplying graphite crystals. This work was supported by the U.S. Department of Energy under grants DE-FG02-07ER46459 and DE-FG02-07ER46453 through the Frederick Seitz Materials Research Laboratory, with use of the Advanced Photon Source supported by DEAC02-06CH11357.

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Materials and Methods

SOM Text

Figs. S1 to S6

References

Movies S1 and S2

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Large-Area Three-Dimensional Molecular Ordering of a Polymer Brush by One-Step Processing

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Rational molecular design and processing, enabling large-area molecular ordering, are important for creating high-performance organic materials and devices. We show that, upon one-step hot-pressing with uniaxially stretched Teflon sheets, a polymer brush carrying azobenzene-containing mesogenic side chains self-assembles into a freestanding film, where the polymer backbone aligns homeotropically to the film plane and the side chains align horizontally. Such an ordered structure forms through translation of a one-dimensional molecular order of the Teflon sheet and propagates from the interface macroscopically on both sides of the film. The resultant wide-area bimorph configuration allows the polymer film to bend rapidly and reversibly when the azobenzene units are photoisomerized. The combination of polymer brushes with hot-pressing and Teflon sheets provides many possibilities in designing functional soft materials.

In biological systems, long-range three-dimensional (3D) molecular ordering, realized by programmed spontaneous assembly of dedicated functional molecules, is crucial to the activities of living organisms. This notion has inspired chemists to develop methods for tailoring elaborate molecular assemblies over a macroscopic length scale, because they are considered promising for creating cutting-edge organic materials and devices (1–3). Examples include shear forces, external fields, and patterned surfaces, which allow 1D macroscopic ordering of certain polymers and liquid crystalline molecules (4–10). However, neither rational molecular design strategy nor processing methodology has yet been developed for 3D ordering of large and/or complex molecules in macroscopic solid materials. We report a serendipitous

discovery that hot-pressing of a certain polymer brush, sandwiched by uniaxially stretched Teflon sheets, affords a freestanding film, in which a 3D molecular order with a remarkably high structural integrity develops widely in a macroscopic length scale (Fig. 1). The width of the film, fabricated by this easy processing, is in principle unlimited.

The polymer brush consists of a polymethacrylate backbone densely grafted to paraffinic side chains containing three azobenzene units that then wrap around the backbone (poly-1, Fig. 1B). Due to a size-exclusion effect, grafted side chains tend to be expelled from the polymer backbone, whereas the backbone itself is likely extended (11–13). Consequently, the polymer brush adopts a cylindrical shape (Fig. 1C). Azobenzene is a frequently used photochromic motif, which undergoes molecular deformations associated with its trans-to-cis and reverse isomerizations upon irradiation with ultraviolet (UV) and visible light, respectively (14–16). As exemplified by the pioneering work of Ikeda and co-workers (17, 18), incorporation of azobenzene units into cross-linked polymer networks affords liquid crystalline elastomers capable of photoactuation. In all successful examples (17–21),

the photomechanical effect relies on a phase transition between liquid crystal and isotropic phases triggered by the azobenzene isomerization. Our interest in the present work was to explore the possibility that polymer brushes, which can accommodate multiple azobenzene units in their densely packed side chains, might show a photomechanical response even without cross-linking. However, a freestanding cast film of poly-1 did not respond to light irradiation. We wondered whether this result might be due to a roughness of the cast film. So, the cast film was sandwiched by Teflon sheets and hot-pressed. The resulting film underwent a reversible bending motion upon alternate irradiation with UV and visible lights. We also noticed that this photomechanical response hardly emerges when the Teflon sheets are arranged in such a way that their drawing directions are orthogonal to one another.

Poly-1 was obtained by free-radical polymerization of 1 (Fig. 1A and fig. S1) (22). In differential scanning calorimetry (DSC), poly-1 showed, on cooling from its isotropic melt, two exothermic peaks at 120° and 103°C (fig. S2), indicating the presence of a mesophase. Synchrotron radiation small-angle x-ray scattering (SAXS) of a bulk sample of poly-1, prepared by heating to its hot melt followed by cooling to the mesophase temperature in a glass capillary (22), displayed clear reflection peaks (Fig. 2A) assignable to a 2D rectangular lattice (space group; $P2_1/a$, Fig. 2B) with lattice parameters a and b of 218(5) and 147(1) Å, respectively. Judging from the magnitudes of a and b , the cylinders of poly-1 most likely deform elliptically to some extent and align parallel to one another without entanglement of their side chains. The SAXS pattern hardly changed when the sample was allowed to solidify by cooling down to room temperature (fig. S3A). Meanwhile, in a wide-angle region (fig. S3B), a broad peak with a d -spacing of 4.4 Å split into two (5.0 and 4.3 Å) upon solidification of the material, suggesting that the azobenzene-containing paraffinic side chains of poly-1 are structured.

When poly-1 was thermally treated by hot-pressing with stretched Teflon sheets, a film

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with unidirectionally aligned polymer lattices on both sides resulted (Fig. 1, D and E). Typically, a cast film of poly-1 (1 mg) from its CHCl_3 solution (1 mg/mL) was sandwiched between two

Teflon sheets (10 cm by 10 cm) in such a way that their drawing directions were parallel to one another (Fig. 2C, left). Then, hot-pressing was conducted under 0.3 MPa at 115°C (mesophase)

after short heating at 130°C for complete melting (Fig. 1D). To realize a large-scale macroscopic ordering, the sample has to be hot-pressed for a certain period (e.g., 1 hour) at the mesophase temperature. The hot-pressed sample was allowed to cool to 25°C, affording a freestanding film with a thickness of 5 μm . When this film was exposed to a synchrotron x-ray beam along a perpendicular direction to the film surface, a through-view 2D SAXS image shown in Fig. 2D (left) was obtained (22), which is characteristic of a rectangular lattice structure ($P2_1/a$). At 25°C, the lattice parameters [$a = 216(4)$ Å, $b = 154(2)$ Å] were identical to those observed for the bulk sample of poly-1 (fig. S3A). The observed diffuse spots in the SAXS image were assigned to the reflections from the (hkl) = (110), (210), (020), and (130) planes. The positions of these spots clearly indicate that the rectangular lattices in the polymer film are oriented unidirectionally in such a way that their b axis is parallel to the drawing direction of the Teflon sheets used for hot-pressing (Fig. 1E and Fig. 2C, left). Most symbolically, the diffuse spots from the (020) plane in Fig. 2D (left) are explicitly observed only in the equatorial direction, which is identical to the drawing direction of the Teflon sheets. Notably, by integration of the 2D SAXS data (Fig. 2D, left, and fig. S4A), we found that the macroscopic order of the polymer lattice develops almost entirely (90%) over the film. The lattice orientation upon hot-pressing is a result of the structural transfer at the poly-1/Teflon sheet interface and most likely develops

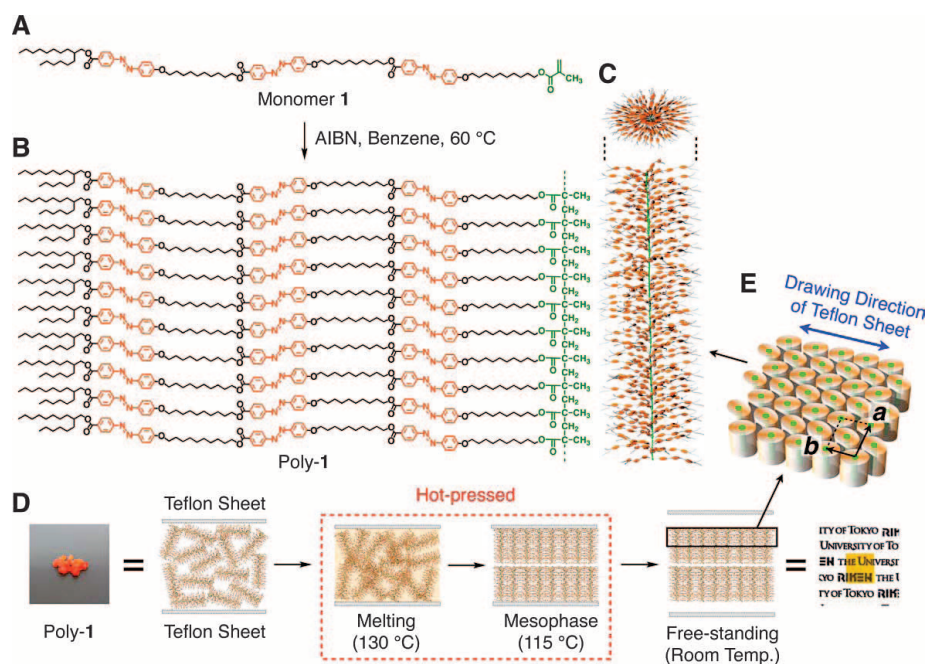
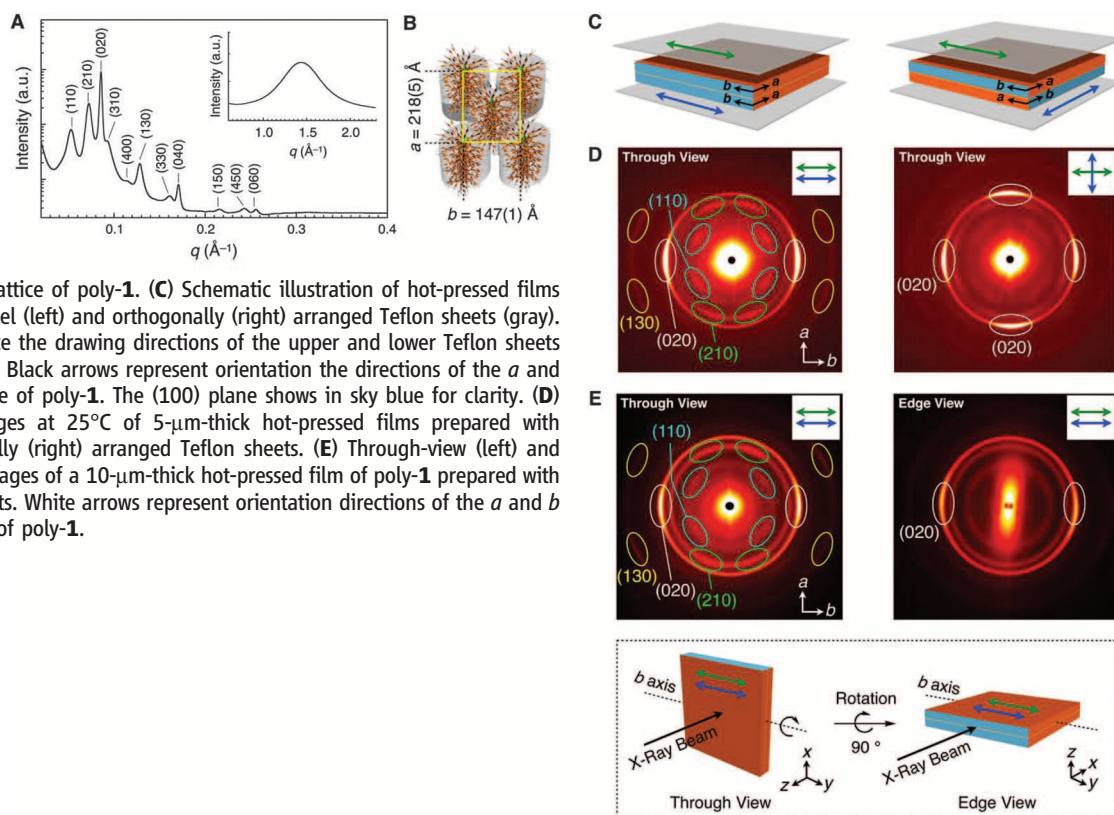


Fig. 1. Molecular formulae of (A) monomer 1 and (B) poly-1. (C) Schematic illustration of poly-1 adopting a cylindrical shape. (D) Schematic illustrations of the procedure for hot-pressing of poly-1 with uniaxially stretched Teflon sheets, together with those of plausible molecular events upon hot-pressing. (E) Schematic illustration of the molecular arrangement in a hot-pressed film of poly-1. Blue arrow denotes the drawing direction of the Teflon sheet for hot-pressing.

Fig. 2. SAXS data of poly-1.

Miller indices are given in parentheses. (A) 1D SAXS pattern of a powdery sample of poly-1 in a capillary, measured at 115°C (mesophase) on cooling from its isotropic melt. (Inset) A scattering profile in a wide-angle region. (B) Schematic illustration of a 2D rectangular lattice of poly-1. (C) Schematic illustration of hot-pressed films (orange) prepared with parallel (left) and orthogonally (right) arranged Teflon sheets (gray). Green and blue arrows denote the drawing directions of the upper and lower Teflon sheets for hot-pressing, respectively. Black arrows represent orientation the directions of the a and b axes of a rectangular lattice of poly-1. The (100) plane shows in sky blue for clarity. (D) Through-view 2D SAXS images at 25°C of 5- μm -thick hot-pressed films prepared with parallel (left) and orthogonally (right) arranged Teflon sheets. (E) Through-view (left) and edge-view (right) 2D SAXS images of a 10- μm -thick hot-pressed film of poly-1 prepared with parallel-arranged Teflon sheets. White arrows represent orientation directions of the a and b axes of a rectangular lattice of poly-1.



toward the inner side of the film equally from both sides. Taking into account the degree of lattice orientation (90%) along with the film thickness (5 μm), the processed film certainly adopts a bimorph configuration, where a 0.4- μm -thick randomly oriented interior layer is sandwiched by 2.3- μm -thick unidirectionally oriented exterior layers (fig. S4A).

To investigate the molecular orientation in the depth direction, we likewise processed a 10- μm -thick film (23) capable of affording an edge-view SAXS image. Although this film displayed an anisotropic through-view 2D SAXS pattern (Fig. 2E, left) analogous to that observed for the 5- μm -thick version (Fig. 2D, left), it showed a lower degree of lattice orientation (51%) (fig. S4B). However, this is reasonable, provided that the film is bimorph, having unidirectionally oriented exterior layers with a thickness comparable to that of the 5- μm -thick version. Fortunately, its edge-view SAXS image revealed that cylinders of poly-1 align homeotropically with respect to the Teflon sheets used for hot pressing (Fig. 1, D and E). Thus, upon stepwise rotation of the film around the *b* axis, the diffuse spots from all the planes except (020) became less intense. When the film plane was set parallel to the direction of the incident x-ray beam (edge-view position), only the (020) spots were observed (Fig. 2E, right). The homeotropic alignment of poly-1 is surprising, because 1D polymers and cylindrical objects such as columnar liquid crystalline assemblies tend to align horizontally.

Further investigations using polarized light demonstrated that not only the polymer lattices but also the azobenzene units of poly-1 in the hot-pressed film align anisotropically over the film plane. Polarized optical microscopy (POM) of the film under crossed polarizers showed a contrast at every 45° on rotation (fig. S5A), giving a completely dark image when the azimuthal angles θ between the polarizing direction of the incident light and the drawing direction of the Teflon sheets were 0° and 90°. Polar plots of the relative absorbance (A_{rel}) of the hot-pressed film at 500 nm versus θ displayed a dichroic feature with a maximum at $\theta = 0^\circ$ and 180° (Fig. 3A). Likewise, in polarized infrared spectroscopy, the stretching vibration bands due to the azobenzene groups of poly-1 showed analogous absorbance ellipsoids (Fig. 3B and fig. S6). From these observations, it is clear that the grafted side chains containing azobenzene units are oriented along the drawing direction of the Teflon sheets (Fig. 1E).

The hot-pressed film of poly-1 rolls up and flattens upon irradiation with UV and visible lights alternately. Thus, when the film (5 mm by 6 mm by 10 μm) was held horizontally and exposed to UV light ($\lambda = 360 \pm 2$ nm) from its upper side, the free edge of the film was quickly lifted up to form a concave shape (Fig. 4A). The photoactuation took place along the orientation direction of the azobenzene units, that is, the

drawing direction of the Teflon sheets used for hot-pressing. This means that the macroscopic processing can determine the bending direction of the film. The bending motion subsided in 10 s to furnish a 2.5-mm displacement at the film edge. Although this concave shape was

maintained after the excitation light was turned off, the film, upon subsequent exposure to visible light ($\lambda = 480 \pm 2$ nm), immediately started to deform toward an opposite direction to recover the initial flat shape within 8 s. The bending and flattening motions are reversible and re-

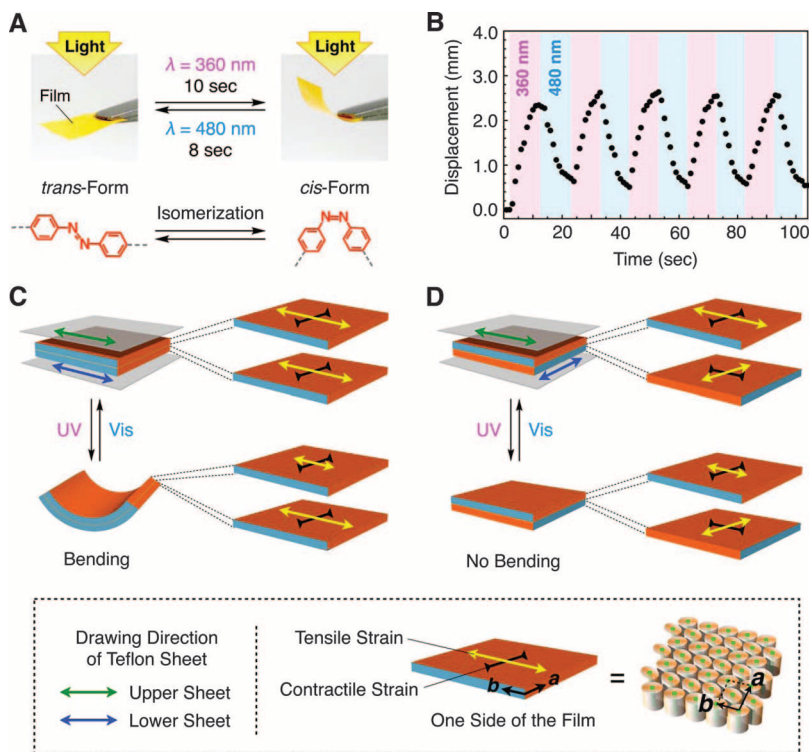
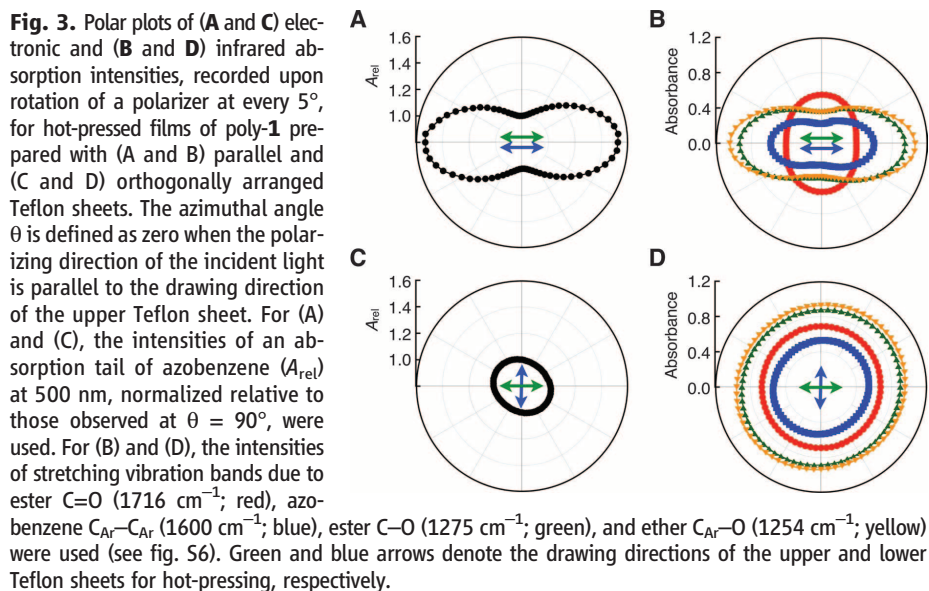


Fig. 4. Photomechanical responses of hot-pressed films of poly-1 prepared with parallel and orthogonally arranged Teflon sheets. (A) Photographs of a hot-pressed film (5 mm by 6 mm by 10 μm) prepared with parallel-arranged Teflon sheets before (left) and after (right) exposure to UV light ($\lambda = 360 \pm 2$ nm). The bent film recovered the initial flat shape upon exposure to visible light ($\lambda = 480 \pm 2$ nm). (B) Time-dependent change in displacement of the free edge of a hot-pressed film prepared with parallel-arranged Teflon sheets upon alternating irradiation with UV and visible lights. (C and D) Schematic illustrations of tensile and contractile strains generated on one side of the films prepared with (C) parallel and (D) orthogonally arranged Teflon sheets.

peatable (Fig. 4B). After 50 repetitions of this bending cycle, the film still preserved its photo-responsiveness, although the displacement was decreased by 85% from that of the initial one (fig. S7). Considering a large absorption coefficient of the azobenzene units, only a limited number of the polymer molecules, located most likely in the photoirradiated surface region of the film, are eligible for photoisomerization. In fact, in a fully bent state of the film after a 10-s exposure to UV light, the content of photoisomerized azobenzene units (1.5%), as determined by ^1H nuclear magnetic resonance spectroscopy, was surprisingly small (24, 25).

Only a limited photomechanical bending motion of the hot-pressed film of poly-1 occurs when the drawing directions of the Teflon sheets, used for hot pressing, are orthogonal to one another (Fig. 2C, right). We found that, upon rotation under crossed polarizers, the hot-pressed film thus obtained does not display any angle-dependent contrast in polarized optical microscopy (fig. S5B). Furthermore, polarized absorption (Fig. 3C) and infrared spectra (Fig. 3D and fig. S6) of this film were not dichroic. Based on these results, we initially thought that the polymer molecules in the film were poorly ordered. However, the film displayed a clear 2D SAXS pattern (Fig. 2D, right) assignable to a rectangular lattice of poly-1, and the degree of lattice orientation was again 90% (fig. S4C). Although these structural profiles are identical to those of the films processed using parallel-arranged Teflon sheets, we observed, in axial as well as equatorial positions, equally intense (020)-diffuse spots (Fig. 2D, right), indicating that the rectangular lattices on both sides of the film are oriented orthogonally to one another (Fig. 2C, right). Why, then, is this film not active photomechanically? After struggling, we found that the hot-pressed films possess particular strains that compete with one another. On cooling poly-1 from its hot-pressed state to room temperature, each oriented polymer lattice expands (with respect to the b axis; $147 \rightarrow 154 \text{ \AA}$) and contracts (with respect to the a axis; $218 \rightarrow 216 \text{ \AA}$) along and across the drawing direction of the Teflon sheet, respectively. These dimensional changes result in generation of tensile and contractile strains, respectively, on both sides of the film (Fig. 4, C and D). When the drawing directions of the Teflon sheets for hot pressing are orthogonal to each other, the tensile and contractile strains generated on one side of the film are orthogonal to those, respectively, on the other (Fig. 4D). We noticed this fact because a square-shaped film of poly-1, processed by orthogonally arranged Teflon sheets, spontaneously rolled up as soon as it was cut into two pieces (fig. S9B). Obviously, this spontaneous deformation is a result of the breakdown of a balance between the competing strains on the front and back sides of the film. Upon trans-to-cis isomerization of poly-1 by UV light, each polymer lattice located on the photoirradiated side of the film contracts along its b axis, there-

by releasing the tensile strain. Consequently, the film tries to bend into a concave shape. However, the parallel strain on the other side of the film is contractile and does not assist the film to bend (Fig. 4D). A totally opposite situation exists in the film obtained with parallel-arranged Teflon sheets, where the tensile and contractile strains generated on one side of the film are parallel to those, respectively, on the other (Fig. 4C). Thus, cutting the film into two pieces does not result in upsetting the balance between these competing strains (fig. S9A). However, when the tensile strain on the photoirradiated side of the film is released by isomerization of poly-1, the competing tensile strain on the other side of the film prevails and assists the film to bend into a concave shape (Fig. 4C). Thus, only a very small degree of the azobenzene isomerization (1.5%) generates a large macroscopic motion of the film.

In the Teflon sheet employed, polytetrafluoroethylene (PTFE) molecules are oriented along the drawing direction. Most likely, this structural information is transferred to the bulk layer of poly-1 at the interface, so that the azobenzene-containing side chains of poly-1 are oriented along the drawing direction of the Teflon sheet. Such a primary side-chain ordering forces the polymer main chain to align homeotropically to the film plane (Fig. 1, D and E). Namely, the role of the polymer brush as a scaffold is to translate a 1D structural order of the Teflon sheet into a 3D molecular order in the processed polymer film. In relation to this notion, we confirmed that the monomer of poly-1 (1, Fig. 1A), likewise hot-pressed, aligns along the drawing direction of the Teflon sheet, affording a lamellar structure with an interlayer distance ($87 \text{ \AA}$) comparable to the molecular length of 1 (fig. S10). The importance of side-chain ordering of poly-1 is supported by the fact that a reference polymer (poly-2) (22) with only a single azobenzene unit in its side chains did not align by hot pressing. Hot-pressing using rubbed polyimide films, instead of stretched Teflon sheets, did not result in orientation of poly-1.

From a general point of view, one-step 3D structural ordering of functional groups in a macroscopic length scale, readily achieved by combining a polymer brush as a scaffold with uniaxially stretched Teflon sheets, provides many new possibilities in the design of advanced functional materials. Of particular interest are bimorph polymer films, where the relative orientation of aligned functional groups on one side to the other can be controlled freely by the arrangement of two Teflon sheets for hot-pressing. The importance of such a bimorph configuration is clearly demonstrated by the fact that the remarkable photomechanical motion occurs only when the polymer lattices on both sides of the bimorph film align parallel to one another. The photomechanical response, thus observed, may also update the common perception on photomechanical soft materials (26–29) that covalent cross-linking of photoactive molecular components is always essential for exerting macroscopic motions (17–21).

References and Notes

- G. M. Whitesides, B. Grzybowski, *Science* **295**, 2418 (2002).
- R. van Hameren *et al.*, *Science* **314**, 1433 (2006).
- S. Zhang *et al.*, *Nat. Mater.* **9**, 594 (2010).
- F. Schneider, H. Knepe, in *Handbook of Liquid Crystals*, D. Demus, J. Goodby, G. W. Gray, H.-W. Spiess, V. Vill, Eds. (Wiley, Weinheim, Germany, 1998), vol. 1, chap. 8.
- L. M. Blinov, in *Handbook of Liquid Crystals*, D. Demus, J. Goodby, G. W. Gray, H.-W. Spiess, V. Vill, Eds. (Wiley, Weinheim, Germany, 1998), vol. 1, chap. 9.
- B. Jérôme, in *Handbook of Liquid Crystals*, D. Demus, J. Goodby, G. W. Gray, H.-W. Spiess, V. Vill, Eds. (Wiley, Weinheim, Germany, 1998), vol. 1, chap. 10.
- J. C. Wittmann, P. Smith, *Nature* **352**, 414 (1991).
- V. K. Gupta, N. L. Abbott, *Science* **276**, 1533 (1997).
- A. M. van de Craats *et al.*, *Adv. Mater.* **15**, 495 (2003).
- R. I. Gearba *et al.*, *Adv. Mater.* **19**, 815 (2007).
- P. Dziezok, K. Fischer, M. Schmidt, S. S. Sheiko, M. Möller, *Angew. Chem. Int. Ed. Engl.* **36**, 2812 (1997).
- M. Zhang, A. H. E. Müller, *J. Polym. Sci. Pol. Chem.* **43**, 3461 (2005).
- S. S. Sheiko, B. S. Sumerlin, K. Matyjaszewski, *Prog. Polym. Sci.* **33**, 759 (2008).
- G. S. Kumar, D. C. Neckers, *Chem. Rev.* **89**, 1915 (1989).
- T. Ikeda, O. Tsutsumi, *Science* **268**, 1873 (1995).
- T. Hugel *et al.*, *Science* **296**, 1103 (2002).
- Y. Yu, M. Nakano, T. Ikeda, *Nature* **425**, 145 (2003).
- T. Ikeda, J.-i. Mamiya, Y. Yu, *Angew. Chem. Int. Ed.* **46**, 506 (2007).
- H. Finkelmann, E. Nishikawa, G. G. Pereira, M. Warner, *Phys. Rev. Lett.* **87**, 015501 (2001).
- M. Camacho-Lopez, H. Finkelmann, P. Palffy-Muhoray, M. Shelley, *Nat. Mater.* **3**, 307 (2004).
- C. L. van Oosten, C. W. M. Bastiaansen, D. J. Broer, *Nat. Mater.* **8**, 677 (2009).
- Materials and methods are available as supporting material on Science Online.
- For the preparation of 10- μm -thick films, a powdery sample of poly-1 (3 mg), instead of its cast film, was hot-pressed (7.8 MPa) under otherwise identical conditions to those for 5- μm -thick films.
- The cis-isomer content was determined from the signal intensities at 6.75 to 6.65 (cis) and 8.17 to 8.05 (trans) parts per million. For comparison, exposure of a CD_2Cl_2 solution of poly-1 ($[1] = 10^{-4} \text{ M}$) to UV light afforded, in the photostationary state, a mixture of the cis- and trans-forms of the azobenzene unit with a mole ratio [cis]/[trans] of 92/8.
- The time-dependent absorption spectral change of poly-1 in the film state, upon successive exposures to UV and visible lights, were obtained by using an optically transparent thin film prepared by spin-coating of its toluene solution (fig. S8).
- In contrast with polymeric systems that require cross-linking of photoactive units, single crystalline materials of some photochromic molecules have been reported to exhibit photoinduced mechanical motions. For example, see (27–29).
- M. Irie, S. Kobatake, M. Horichi, *Science* **291**, 1769 (2001).
- S. Kobatake, S. Takami, H. Muto, T. Ishikawa, M. Irie, *Nature* **446**, 778 (2007).
- H. Koshima, N. Ojima, H. Uchimoto, *J. Am. Chem. Soc.* **131**, 6890 (2009).
- We thank D. Hashizume (RIKEN) for his helpful discussion on x-ray analysis. N.H. thanks the RIKEN Junior Research Associate Fellowship and the Japan Society for the Promotion of Science Young Scientist Fellowship. T.F. is thankful to the Ministry of Education, Culture, Sports, Science, and Technology, Japan, for financial support (21350108). The synchrotron x-ray diffraction experiments were performed at the BL45XU in the SPring-8 with the approval of the RIKEN SPring-8 Center (proposal 20090053).

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6005/808/DC1
Materials and Methods
Figs. S1 to S10
References

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Broken-Symmetry States in Doubly Gated Suspended Bilayer Graphene

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The single-particle energy spectra of graphene and its bilayer counterpart exhibit multiple degeneracies that arise through inherent symmetries. Interactions among charge carriers should spontaneously break these symmetries and lead to ordered states that exhibit energy gaps. In the quantum Hall regime, these states are predicted to be ferromagnetic in nature, whereby the system becomes spin polarized, layer polarized, or both. The parabolic dispersion of bilayer graphene makes it susceptible to interaction-induced symmetry breaking even at zero magnetic field. We investigated the underlying order of the various broken-symmetry states in bilayer graphene suspended between top and bottom gate electrodes. We deduced the order parameter of the various quantum Hall ferromagnetic states by controllably breaking the spin and sublattice symmetries. At small carrier density, we identified three distinct broken-symmetry states, one of which is consistent with either spontaneously broken time-reversal symmetry or spontaneously broken rotational symmetry.

In mono- and bilayer graphene, an unconventional quantum Hall effect arises from the chiral nature of the charge carriers in these materials (1–6). In monolayers, the sequence of Hall plateaus is shifted by a half integer, and each Landau level (LL) is fourfold degenerate due to spin and valley degrees of freedom. The latter valley degree of freedom refers to the conduction and valence bands in single-layer graphene forming conically shaped valleys that touch at two inequivalent Dirac points. In bilayer graphene, an even richer picture emerges in the lowest LL caused by an additional degeneracy between the zeroth and first orbital LLs, giving rise to an eightfold degeneracy. Systems in which multiple LLs are degenerate give rise to broken-symmetry states caused by electron-electron interactions (7). Such interaction-induced broken-symmetry states in the lowest LL of bilayer graphene have been theoretically predicted (8, 9) and experimentally observed (10–14), but the nature of their order parameters is still debated. For example, two possible order parameters that have been suggested for the gapped phase at filling factor $\nu = 0$ at large magnetic fields are either layer or spin polarization (9). Moreover, recent theoretical studies predict that even in the absence of external magnetic and electric fields, the parabolic dispersion of bilayer graphene can lead to broken-symmetry states that are induced by interactions among the charge carriers (15–22). In this work, we map out the various broken-symmetry states as a function of external magnetic and electric field. The nature of these phases can be deduced by investigating their stability under the variation of these symmetry-breaking fields.

The observation of broken-symmetry states driven by effects of interaction is hampered by the presence of disorder and requires high sample quality. We have developed a method in which bilayer graphene is suspended between a top gate electrode and the substrate. This approach allows us to combine the high quality of suspended devices with the ability to independently control electron density and perpendicular electric field $E_{\perp}$.

A false-color scanning electron micrograph of a typical device is shown in Fig. 1A. The suspended graphene (red) is supported by gold contacts (yellow). Suspended top gates (blue) can be designed to cover only part of the graphene (left device) or to fully overlap the entire flake (right device), including part of the contacts. We have investigated both types of devices, which show similar characteristics. The fabrication of such two-terminal devices is detailed in the Supporting Online Material (23) and is schematically illustrated in Fig. 1A. To improve sample quality, we current anneal (24, 25) our devices in vacuum at 4 K before measurement.

The high quality of our suspended flakes is evident from the dependence of resistance versus applied electric field at zero magnetic field. As theoretically predicted (26–28) and experimentally verified in transport (29, 30) and optical (30–32) experiments, an applied perpendicular electric field $E_{\perp}$ opens a gap $\Delta \sim dE_{\perp}/k$ in the otherwise gapless dispersion of bilayer graphene, as schematically shown in the lower left inset to Fig. 1B. Here, d is the distance between the graphene sheets and k is a constant that accounts for imperfect screening of the external electric field by the bilayer (19, 26, 28). The conductance of our suspended bilayer graphene sheet at 100 mK as a function of back (V_b) and top gate (V_t) voltage is shown in Fig. 1B. The opening of a field-induced band gap is apparent from the decreased conductance at the charge neu-

trality point with increasing applied electric field $E_{\perp} = (\alpha V_t - \beta V_b)/2e\epsilon_0$. Here, α and β are the gate coupling factors for V_t and V_b , respectively, which we determine from the Landau fan in the quantum Hall regime, e is the electron charge, and ϵ_0 is the vacuum permittivity.

The presence of an energy gap is also illustrated by the line cut in Fig. 1C, which shows the resistance at constant $E_{\perp}$ as a function of total density $n = (\alpha V_t + \beta V_b)$. By varying the density, the resistance can be changed by a factor 10^3 . Line traces of the maximum sheet resistance as a function of $E_{\perp}$ at various temperatures (Fig. 1D) illustrate the exponential dependence of the resistance on electric field, as well as a decrease in resistance with temperature. The sheet resistance at 100 mK increases by more than a factor of 2×10^3 (from 8 kilohm per square to 20 megohm per square) for $E_{\perp}$ between 20 and 90 mV/nm. Compared to previous measurements of dually gated graphene bilayers embedded in a dielectric (29), our measurements show an increase by a factor of 10^3 in resistivity at the same electric field, which is the result of the high quality of our flakes. The unexpected finding of a nonmonotonous dependence of the resistance at small applied electric field will be discussed below. The oscillations in the conductance traces shown in Fig. 1C are repeatable and result from mesoscopic conductance fluctuations. A detailed description of the temperature dependence of the resistance and of these fluctuations is presented in figs. S1 and S2 (23).

The evolution of different LLs in our samples at nonzero magnetic field was revealed by measuring conductance as a function of $E_{\perp}$. The two-terminal conductance as a function of density and electric field at various different magnetic fields (Fig. 2, A to D) shows the previously reported eightfold degeneracy of the lowest LL, which is fully lifted because of electron-electron interactions (10, 11, 14, 23). Plateaus at $\nu = 0, \pm 1, \pm 2, \pm 3$ can be identified by their slope in a fan diagram. Vertical line cuts that correspond to a constant filling factor show that the conductance is quantized except at particular values of electric field, denoted by stars or dots in Fig. 2, A to D. The $\nu = 0$ state is quantized except near two values of the applied electric field (marked with dots). This value of electric field increases as the magnetic field B increases. The $\nu = \pm 2$ state is quantized for all electric fields except near $E_{\perp} = 0$; finally, the $\nu = \pm 4$ state is quantized for all electric fields.

A qualitative understanding of this phenomenology can be gained from a simplified scheme of the LL energies as a function of electric field (Fig. 2E, gray lines) for nonzero magnetic field. We start by neglecting the breaking of the orbital degree of freedom so that the LLs are each doubly degenerate (6). We assume, in accordance with theoretical predictions, that at $E_{\perp} = 0$ only the spin degeneracy is lifted, giving rise to a spin-polarized $\nu = 0$ quantum Hall ferromagnetic state (9, 19). In (23), we have detailed

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how our experimental data support this assumed sequence of degeneracy breaking. In the lowest LL, the layer and valley index are equivalent (6) so that an electric field that favors one of the layers directly controls the valley-pseudospin. As a result, LLs of quantum number U (upper layer) or L (lower layer) are expected to have different slopes in electric field. At several points, marked by dots or stars in the schematic of Fig. 2E, LL crossings occur. A LL crossing is seen at $E_{\perp} = 0$ for both $\nu = 2$ and $\nu = -2$, and two LL crossings are seen for $\nu = 0$ at nonzero $E_{\perp}$. We hypothesize that these crossings are responsible for the increased conductance in our transport experiments (33). Figure 2E shows the LL energy, whereas we have direct control over the density of the bilayer rather than its chemical potential. However, on a quantum Hall plateau, the chemical potential lies between two LL energies, which enables us to relate our scheme in Fig. 2E to our transport data as detailed below [see fig. S3 (23)].

The LL crossings at zero average carrier density are marked by dots in Fig. 2. In our proposed scheme, these transitions separate a spin-polarized $\nu = 0$ state at low electric fields (I) from two layer-polarized $\nu = 0$ states at large electric fields (II) of opposite layer polarization. The line cut in Fig. 2C at constant filling factor shows that insulating $\nu = 0$ states are well developed at zero electric field and at large electric fields, but

the crossover between these states is marked by a region of increased conductance. The experimentally observed large resistance of the phase at $E_{\perp} = 0$ (10, 11) is at variance with the theoretical prediction of percolating edge modes (34) in the case of a spin-polarized $\nu = 0$ state. A possible reason for this discrepancy is the mixing of counter-propagating edge modes and subsequent opening of a gap (35).

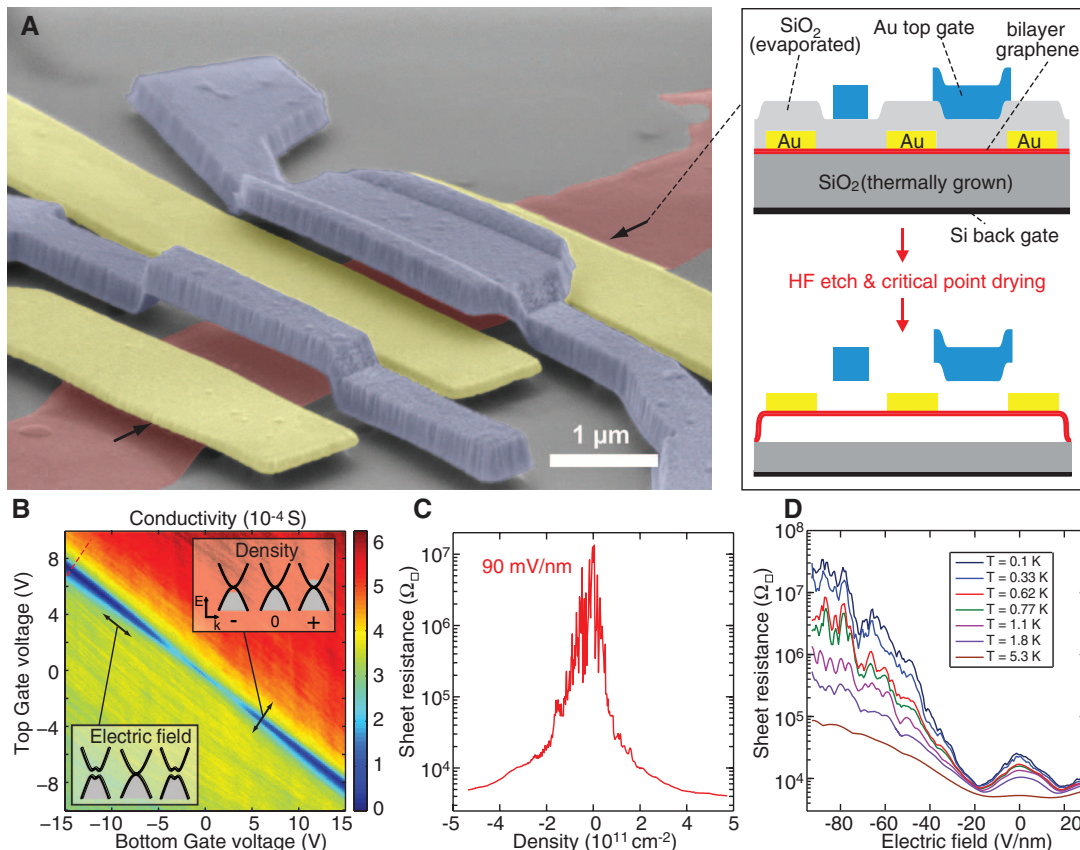
The LL crossings at $\nu = \pm 2$ near zero electric field are marked by stars in Fig. 2. An example of such a crossing is apparent in the vertical line cut shown in Fig. 2B. There, the conductance at $\nu = \pm 2$ increases near zero electric field, which is explained in our scheme by a crossing between two LLs of identical spin polarization but opposite layer polarization. The consequence of these crossings is that only $\nu = 0$ and $\nu = \pm 4$ states occur at zero electric field, whereas layer-polarized $\nu = \pm 2$ states emerge only at finite electric field, as is apparent in the horizontal line cuts of Fig. 2B.

Assuming that the $\nu = \pm 1$ and ± 3 states are partially layer polarized (9), the electric field should also induce a splitting at these filling factors (inset to Fig. 2E) that is, however, more fragile and only seen at higher magnetic fields. Our simple model predicts that the $\nu = \pm 3$ state will have a crossing at zero electric field, and we attribute the increase in conductance at $E_{\perp} = 0$ in the left line cut in Fig. 2D to be a signature of this crossing. A line cut at $\nu = \pm 3$ at even higher

magnetic field showing a better quantization is shown in fig. S4 (23). The $\nu = \pm 1$ state is expected to have three crossings: one at zero electric field and two near the same nonzero electric field at which the transition between the $\nu = 0$ states is observed. All three of these crossings are apparent from the regions of increased conductance in the right line cut in Fig. 2D. The observation that $\nu = \pm 1$ and ± 3 states are enhanced with electric field is an indication of their partial layer polarization. Theory nonetheless predicts that $\nu = \pm 1, \pm 2, \pm 3$ should also be seen at $E_{\perp} = 0$ (9). However, the predicted energy gaps of these states are expected to be considerably smaller than that of $\nu = 0$ and hence visible only at large magnetic field. Indeed, for $B > 4$ T, these broken-symmetry states at $E_{\perp} = 0$ can also be observed in our data [see fig. S4 (23)].

When we examine the conductance at the charge neutrality point as a function of electric and magnetic field (Fig. 3A) in more detail, we observe that regions of high conductance mark the transition between the low- and high-field $\nu = 0$ states (highlighted with dots in Fig. 2). The transition moves out to larger electric fields as the magnetic field is increased, which implies that phase I is stable at large magnetic fields and is destabilized by an electric field. This observation is also consistent with our energy diagram (Fig. 2E), which predicts that the $\nu = 0$ LL crossing will move out to higher electric fields when the

Fig. 1. (A) False-color scanning electron micrograph of a bilayer graphene flake suspended between Cr/Au electrodes with suspended Cr/Au top gates. A schematic of a cross section along a line marked by the two arrows is shown on the right, including a brief depiction of the sample fabrication. We used a three-step electron-beam lithography process in which Cr/Au contacts are first fabricated on the bilayer graphene, followed by deposition of a SiO_2 spacer layer on top of the flake and finally the structure of a Cr/Au topgate above the SiO_2 layer. Subsequent etching of part of the SiO_2 leaves the graphene bilayer suspended between the top and bottom gate electrodes. **(B)** Conductance map as function of top and bottom gate voltage at $T = 100$ mK of a suspended bilayer graphene flake. **(C)** Line trace along the dashed lines in (B) showing the sheet resistance as a function of charge carrier density at constant electric field. **(D)** Traces of the maximal sheet resistance at the charge neutrality point as a function of applied electric field at different temperatures.



magnetic field increases. The increased stability of this phase at higher magnetic fields reconfirms our initial assumption that phase I is spin polarized. In contrast, the phase at large electric fields (phase II) is stabilized by an electric field, consistent with it being layer polarized.

The dependence of the transition region between the two phases on electric and magnetic field is shown more clearly in Fig. 3B, where we have extracted the maximum conductance in Fig. 3A for all positive values of electric field. At large magnetic field, the transition line is linear and is independent of sample quality and temperature (we have investigated a total of five samples at temperatures between 100 mK and 5 K). At magnetic fields below about 2 T, however, the transition line is linear only in the highest-quality sample and at low temperatures (inset to Fig. 3A and dashed line in Fig. 3B). A surprising observation is that the extrapolation of the linear dependence from high B down to $B = 0$ results in a crossing at a nonzero electric field, E_{off} . Moreover, the slope and E_{off} that characterize the large B behavior seem to have no systematic dependence on sample quality or temperature T (Fig. 3, C and D). The conductance at the transition between the $\nu = 0$ phases decreases both with increasing B as well as with decreasing T [see fig. S5 (23)]. Such behavior is qualitatively expected given that LL mixing at these crossing points can lead to the opening of gaps in the spectrum.

To further elucidate the nature of the transition, we investigated the role of the Zeeman energy E_z across the $\nu = 0$ transition in detail. We tilted the sample with respect to the magnetic field, which altered E_z but left all interaction-dependent energy scales the same. Figure 3B shows both measurements under tilted magnetic field (green line) and data taken in purely perpendicular field (blue line). The two transition lines have similar slopes, which indicates that E_z is negligible and the transition predominantly depends on the perpendicular component of the magnetic field, underscoring that exchange effects and the LL degeneracies play an important role. However, this explanation does not account for the $B = 0$ offset.

The crossover between two phases with the application of an electric field has also been predicted theoretically by Gorbar *et al.* (36) and Nandkishore and Levitov (19). Gorbar *et al.* (36) predict a transition from a quantum Hall ferromagnetic state at low electric fields to an insulating state dominated by magnetic catalysis with a slope of about $2 \text{ mV nm}^{-1} \text{ T}^{-1}$. Nandkishore and Levitov (19) predict a transition from a quantum Hall ferromagnetic state to a layer insulating state at large electric fields, with a slope of $34 \text{ mV nm}^{-1} \text{ T}^{-1}$. Our measured slope is about $11 \text{ mV nm}^{-1} \text{ T}^{-1}$. A qualitative comparison between the experimentally obtained values and the theory is difficult because of the lack of knowledge of the screening that the bilayer provides to the applied electric field once LLs are formed.

A notable feature seen in the inset to Fig. 3A and in Fig. 3B is that the transition line appears to

have a nonzero offset in electric field, $E_{\text{off}} \approx 20 \text{ mV/nm}$. The $B = 0$ offset coincides with the extrapolated one from high fields when the sample quality is high and the temperature is low. This result suggests that the transition from a spin- to a layer-polarized phase persists all the way down to $B = 0$, but careful measurements near $B = 0$ and $E_{\perp} = 0$ (Fig. 4A) show that this conclusion is incorrect. We will discuss possible origins for E_{off} in the remainder of this manuscript.

The conductance close to the charge neutrality point at small electric and magnetic fields is shown in Fig. 4A. The conductance at $B = 0$ and $E_{\perp} = 0$ exhibits a local minimum and increases upon increase of $E_{\perp}$ and B . The electric

field value at which the conductance reaches a maximum coincides with E_{off} . Together with observation of a maximum of the conductance at a finite value $B_{\text{off}} = \pm 50 \text{ mT}$, our observations suggest that neither the spin-polarized phase I nor the layer-polarized phase II extend down to $B = 0$ and $E_{\perp} = 0$. Instead, our measurements are consistent with the presence of a third phase at small electric and magnetic fields. The electric field dependence of the resistance is at variance with simple calculations of the band structure of bilayer graphene. In a tight binding model, bilayer graphene is expected to evolve from a gapless semimetal to a gapped semiconductor whose gap magnitude depends monotonically

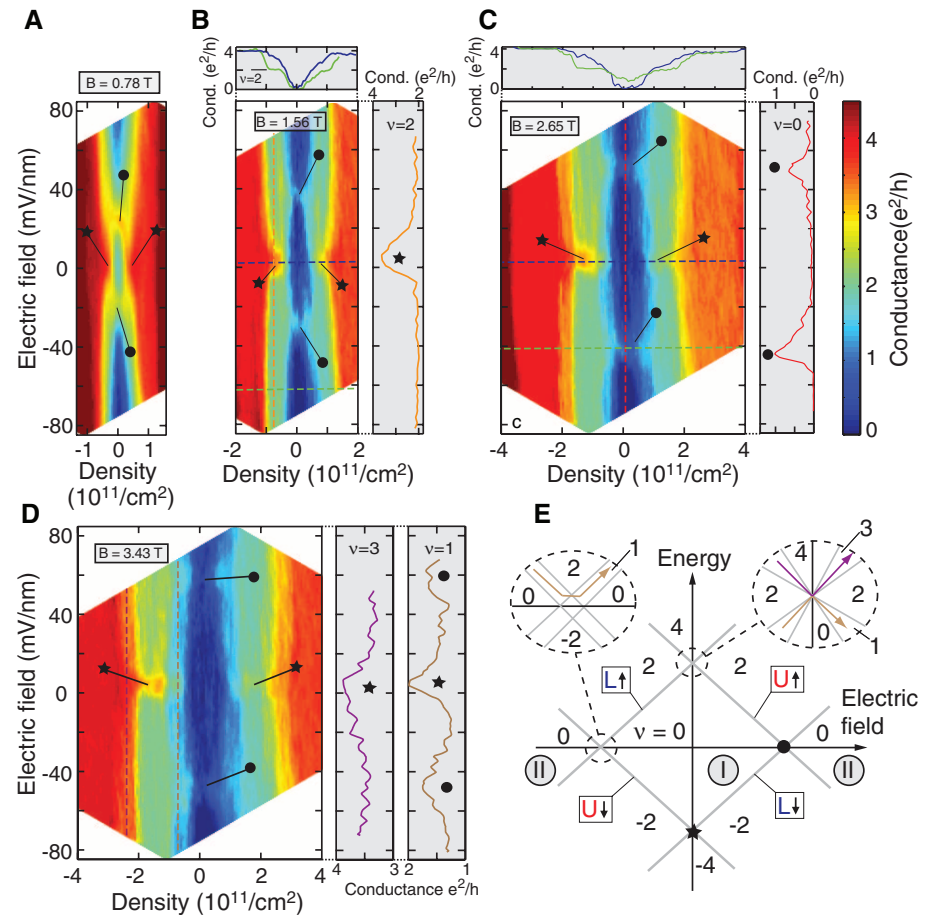


Fig. 2. (A to D) Maps of the conductance in units of e^2/h as a function of applied electric field $E_{\perp}$ and density n at various constant magnetic fields at $T = 60 \text{ mK}$. Line traces are taken from the data along the dashed lines. Horizontal line traces correspond to cuts at constant electric field; vertical line cuts correspond to cuts along constant filling factor. In (B), the orange line cut corresponds to the electric field dependence of $\nu = 0$ and the vertical line cuts to the density dependence of the conductance at two constant electric fields showing the emergence of $\nu = 2$ at large electric fields. In (C), the red vertical line cut corresponds to the electric field dependence of $\nu = 0$. The horizontal line cuts in green and blue show the density dependence of the conductance at two different electric fields showing the suppression of the $\nu = 0$ state at a finite electric field. In (D), the brown (violet) line cut shows the conductance as function of electric field at $\nu = 1$ ($\nu = 3$). (E) Schematic diagram of the energetic position of the lowest LL octet (gray lines) as a function of $E_{\perp}$. The quantum numbers U , L , $\uparrow$, and $\downarrow$ of the LLs are indicated in the squares. The respective filling factors ν are indicated in black numbers. The LLs in the main scheme are all doubly degenerate in orbital quantum numbers. The effect of the electric field on the orbital LLs is shown in the two insets. The two different $\nu = 0$ states are marked with Roman numerals. In all images, the crossing of LLs at zero electric field is marked with a star and the crossing at zero energy is marked with a dot.

on electric field (26). However, our measurements show that the conductance does not decrease monotonically with electric field, instead exhibiting a local maximum at about ± 20 mV/nm

(Fig. 4B). The maximum resistance at $B = 0$ and close to zero carrier density as a function of $E_{\perp}$ for different temperatures increases as T decreases [see Fig. 1D and fig. S8 (23)], reaching 20 kilohm

at the lowest temperature of 100 mK. It therefore strongly suggests that the neutral bilayer graphene system is already gapped at $E_{\perp} = 0$ and $B = 0$ and that fields larger than E_{off} or B_{off} transfer the system into the layer- or spin-polarized phases (Fig. 4A). In Fig. 4B, the dependence of the conductance as a function of density and electric field at $B = 0$ is shown. Also in this case, a minimum of the conductance at small electric field and density can be discerned, indicating that the spontaneous phase is unstable away from the charge neutrality point. Further experimental evidence for phase III in different samples is given in figs. S6 and S7 (23). The temperature dependence at the crossover field of $E_{\perp} = \pm 20$ mV/nm is weak (Fig. 1D), consistent with the closure of a gap. Our observations cannot be explained with known single-particle effects, as detailed in (23).

It has been pointed out theoretically (15–22) that electron-electron interactions can open a spontaneous gap in bilayer graphene near the charge neutrality point at zero electric and magnetic fields. Several different phases have been proposed, and our experimental observations considerably restrict the various possible theoretical explanations to two prevailing theories. The first theory (19) predicts a phase diagram very similar to what we measure. Within this theory, phase III is associated with an anomalous quantum Hall insulating state that spontaneously breaks time-reversal symmetry. This state is characterized by an antiferromagnetic ordering in the pseudospin (layer). In this phase, electrons from one valley occupy one of the layers, whereas electrons from the other valley occupy the other layer. An important feature of this state is that it supports topologically protected current-carrying edge modes and is therefore predicted to have a finite conductance. This prediction is consistent with our observations of a nondiverging resistance at $B = 0$ and $E_{\perp} = 0$ as T is lowered [see fig. S8 (23)]. Although our observed phase diagram agrees qualitatively with that predicted in (19), we find a quantitative disagreement between the measured and predicted E and B

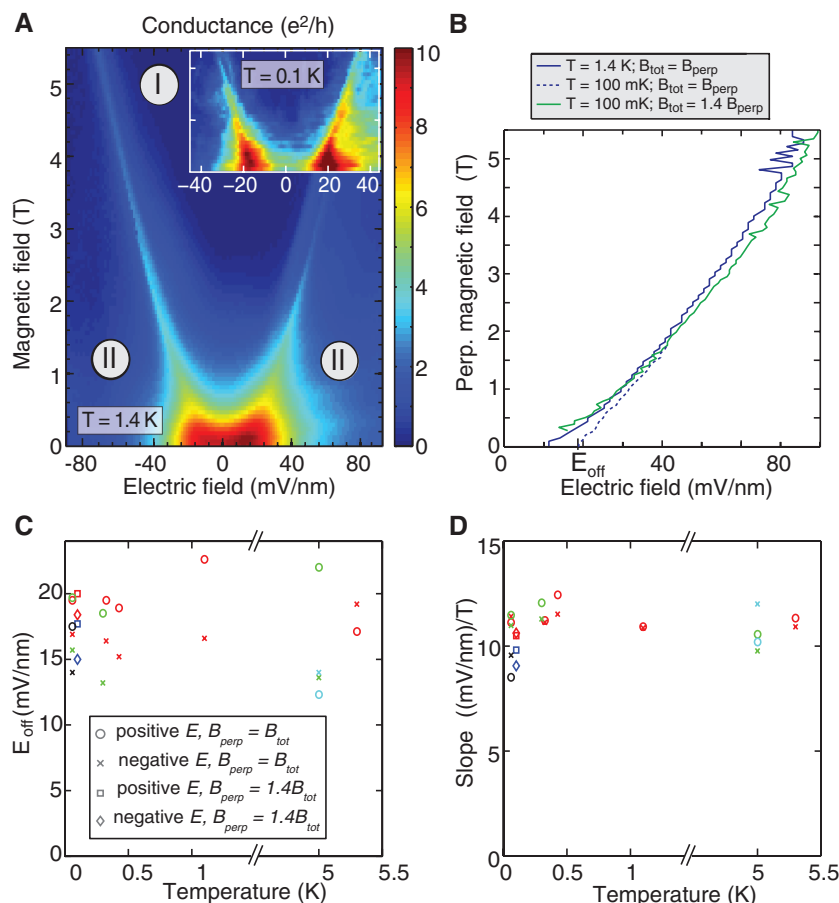


Fig. 3. (A) Conductance in units of e^2/h as a function of applied electric and magnetic fields at zero average carrier density at 1.4 K. The transition between different $\nu = 0$ states is characterized by a region of increased conductance. (Inset) Measurement taken at 100 mK when the sample has been tilted with respect to the magnetic field, plotted against the perpendicular component of the magnetic field after thermal cycling. The color scale of the inset ranges from 0 (blue) to 4.5 (red) e^2/h . The y axis of the inset ranges from 0 to 1.3 T. (B) Comparison of the slope of the transition line when the sample is perpendicular to the magnetic field and at a 45° angle. (C and D) Comparison of the E_{off} and high-magnetic field slope of the transition line for different temperatures. Different colors correspond to different samples.

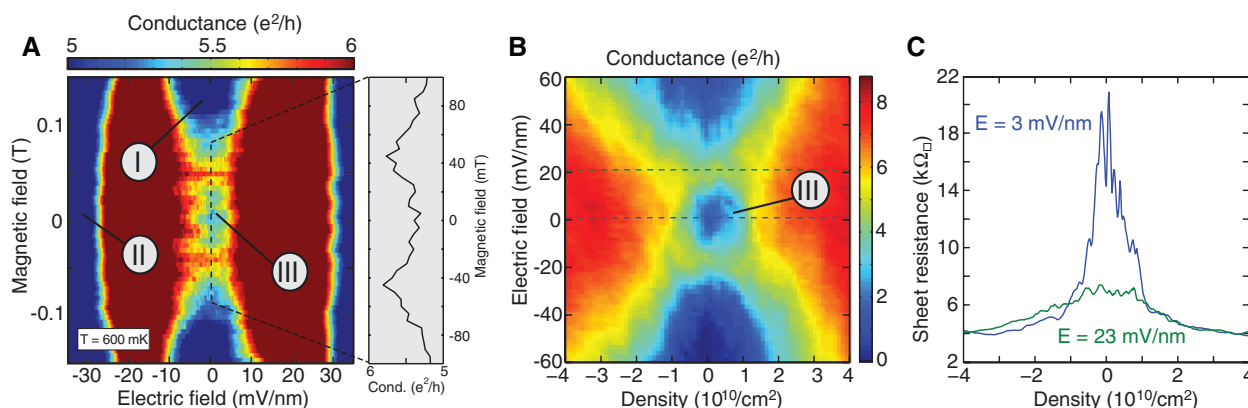


Fig. 4. Experimental evidence of a spontaneous gap in suspended bilayer graphene. (A) Detailed view of the conductivity at small electric and magnetic fields and zero average carrier density. The color scale has been restricted to between 5 and 6 e^2/h to highlight the observed effect. (B) Conductivity as a

function of electric field and density at zero magnetic field. (C) Two linecuts showing the resistivity at $E = 0$ and E_{off} are also shown. The scans in (B) and (C) were taken after thermal cycling of the sample, hence the difference in the minimal conductance at zero magnetic and electric field with respect to (A).

transition values. We find that the magnetic field at which the spontaneous phase breaks down is about 50 mT, an order of magnitude smaller than the theoretically predicted value of 500 mT (19). An applied electric field of about $E_{\text{off}} = 20$ mV/nm quenches the spontaneous gap, compared to the predicted value of 26 mV/nm for the screened electric field. Notably, we need to scale our measured E_{off} by the screening constant k , which means that our values differ by roughly a factor $k = 3$ from the theory (19). A second prevailing theory for the observed behavior stems from symmetry breaking, which results in the lowering of the density of states at the charge neutrality point. One such example is the nematic state that stems from the breaking of rotational symmetry due to electron-electron interactions. This phase has been predicted to lead to a decreased density of states at the charge neutrality point due to the breaking of the system into two Dirac cones (21, 22), consistent with our measured decrease in conductance at small densities. Further experimental support for the above two scenarios is given in (13) and elaborated in (23), where it is shown that known single-particle effects cannot explain the observed behavior.

References and Notes

1. K. S. Novoselov *et al.*, *Nature* **438**, 197 (2005).
2. Y. B. Zhang, Y. W. Tan, H. L. Stormer, P. Kim, *Nature* **438**, 201 (2005).
3. K. S. Novoselov *et al.*, *Nat. Phys.* **2**, 177 (2006).
4. A. K. Geim, K. S. Novoselov, *Nat. Mater.* **6**, 183 (2007).
5. A. H. Castro Neto, F. Guinea, N. M. R. Peres, K. S. Novoselov, A. K. Geim, *Rev. Mod. Phys.* **81**, 109 (2009).
6. E. McCann, V. I. Fal'ko, *Phys. Rev. Lett.* **96**, 086805 (2006).
7. K. Yang *et al.*, *Phys. Rev. Lett.* **72**, 732 (1994).
8. M. Ezawa, *J. Phys. Soc. Jpn.* **76**, 094701 (2007).
9. Y. Barlas, R. Côté, K. Nomura, A. H. MacDonald, *Phys. Rev. Lett.* **101**, 097601 (2008).
10. B. E. Feldman, J. Martin, A. Yacoby, *Nat. Phys.* **5**, 889 (2009).
11. Y. Zhao, P. Cadden-Zimansky, Z. Jiang, P. Kim, *Phys. Rev. Lett.* **104**, 066801 (2010).
12. W. Bao *et al.*, <http://arxiv.org/abs/1005.0033v1> (2010).
13. J. Martin, B. E. Feldman, R. T. Weitz, M. T. Allen, A. Yacoby, <http://arxiv.org/abs/1009.2069v1> (2010).
14. C. R. Dean *et al.*, *Nat. Nanotech.* **5**, 733 (2010).
15. F. D. M. Haldane, *Phys. Rev. Lett.* **61**, 2015 (1988).
16. S. Raghu, X.-L. Qi, C. Honerkamp, S.-C. Zhang, *Phys. Rev. Lett.* **100**, 156401 (2008).
17. H. Min, G. Borghi, M. Polini, A. H. MacDonald, *Phys. Rev. B* **77**, 041407 (2008).
18. R. Nandkishore, L. S. Levitov, <http://arxiv.org/abs/0907.5395v1> (2009).
19. R. Nandkishore, L. S. Levitov, <http://arxiv.org/abs/1002.1966v1> (2010).
20. F. Zhang, H. Min, M. Polini, A. H. MacDonald, *Phys. Rev. B* **81**, 041402 (2010).
21. O. Vafek, K. Yang, *Phys. Rev. B* **81**, 041401 (2010).
22. Y. Lemonik, I. L. Aleiner, C. Toke, V. I. Fal'ko, <http://arxiv.org/abs/1006.1399v1> (2010).
23. Supporting online material is available on *Science* Online.
24. J. Moser, A. Barreiro, A. Bachtold, *Appl. Phys. Lett.* **91**, 163513 (2007).
25. K. I. Bolotin *et al.*, *Solid State Commun.* **146**, 351 (2008).
26. E. McCann, *Phys. Rev. B* **74**, 161403(R) (2006).
27. E. V. Castro *et al.*, *Phys. Rev. Lett.* **99**, 216802 (2007).
28. H. K. Min, B. Sahu, S. K. Banerjee, A. H. MacDonald, *Phys. Rev. B* **75**, 155115 (2007).
29. J. B. Oostinga, H. B. Heersche, X. L. Liu, A. F. Morpurgo, L. M. K. Vandersypen, *Nat. Mater.* **7**, 151 (2008).
30. Y. B. Zhang *et al.*, *Nature* **459**, 820 (2009).
31. A. B. Kuzmenko *et al.*, *Phys. Rev. B* **79**, 115441 (2009).
32. K. F. Mak, C. H. Lui, J. Shan, T. F. Heinz, *Phys. Rev. Lett.* **102**, 256405 (2009).
33. Y. P. Shkolnikov, E. P. De Poortere, E. Tutuc, M. Shayegan, *Phys. Rev. Lett.* **89**, 226805 (2002).
34. D. A. Abanin *et al.*, *Phys. Rev. Lett.* **98**, 196806 (2007).
35. E. Shimshoni, H. A. Fertig, G. V. Pai, *Phys. Rev. Lett.* **102**, 206408 (2009).
36. E. V. Gorbar, V. P. Gusynin, V. A. Miransky, *Phys. Rev. B* **81**, 155451 (2010).
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Efficient Atmospheric Cleansing of Oxidized Organic Trace Gases by Vegetation

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The biosphere is the major source and sink of nonmethane volatile organic compounds (VOCs) in the atmosphere. Gas-phase chemical reactions initiate the removal of these compounds from the atmosphere, which ultimately proceeds via deposition at the surface or direct oxidation to carbon monoxide or carbon dioxide. We performed ecosystem-scale flux measurements that show that the removal of oxygenated VOC via dry deposition is substantially larger than is currently assumed for deciduous ecosystems. Laboratory experiments indicate efficient enzymatic conversion and potential up-regulation of various stress-related genes, leading to enhanced uptake rates as a response to ozone and methyl vinyl ketone exposure or mechanical wounding. A revised scheme for the uptake of oxygenated VOCs, incorporated into a global chemistry-transport model, predicts appreciable regional changes in annual dry deposition fluxes.

Large quantities of nonmethane volatile organic compounds (NMVOCs) enter the atmosphere via biogenic, pyrogenic, and anthropogenic sources. The annual production of NMVOCs [~ 1200 to 1350 teragrams of carbon (TgC)/year] probably exceeds that of methane and carbon monoxide (CO) (~ 500 TgC/year each) (1, 2). Together, these gases fuel tropospheric chemistry. Oxidation of NMVOCs leads to the formation of aerosols (3–5) and modulates the oxidation ca-

capacity of the atmosphere (6), creating important climate feedbacks (7). One large uncertainty in constraining budgets of NMVOCs is the amount of deposition to vegetation, which acts as a major source and sink for organic trace gases on a global scale. This has consequences for constraining secondary species produced in the gas phase, which will either oxidize to CO and carbon dioxide (CO₂), condense onto or form organic aerosol (OA) and be rained out, or directly deposit to the surface

via dry and wet deposition. Two recent bottom-up assessments of the tropospheric organic aerosol budget (1, 3), on the basis of different assumptions for wet and dry deposition of organic vapors, resulted in different predictions of global production rates for secondary organic aerosol (SOA).

Dry deposition schemes parameterize the deposition flux according to

$$F = v_d \times C \quad (1)$$

where F represents the deposition flux, C is the ambient concentration, and v_d is the deposition velocity. Deposition velocities are usually treated in analogy to Ohm's Law, where v_d can be expressed as three resistances in series:

$$v_d = \frac{1}{R_a + R_b + R_c} \quad (2)$$

R_a represents the aerodynamic resistance above the surface and has the same value for all constituents. The term R_b is the quasi-laminar resistance to transport through the thin layer of air in

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contact with surface elements and varies with the diffusivity of a substance. Standard micrometeorological methods and modeling approaches are available to calculate R_a and R_b (8). R_c represents the resistance to uptake by surface elements and has been extensively parameterized for ozone (O_3) and sulfur dioxide (SO_2) (9). Because O_3 immediately decomposes inside plants by reduction, a relative measure of reactivity ($f_0 = 0$ to 1) accounts for its loss (9). Stomatal resistance (R_s) primarily controls the deposition of highly reactive compounds (10). Because of the lack of observational constraints, accounting for the uptake of organic gases occurs in analogy to O_3 and SO_2 solely on the basis of their physiochemical properties (solubility and reactivity) in the mesophyll. As a consequence, all models treat NMVOCs as nonreactive or only slightly reactive species ($f_0 = 0$ to 0.1), leading to large estimates of R_c (10).

In this report, we combine field observations with laboratory experiments and transport modeling in order to investigate the influence of vegetation on the deposition of oxygenated VOCs (oVOCs). oVOCs represent the most abundant class of organic carbon and profoundly affect the chemical composition in Earth's oxidizing atmosphere. In six field experiments across a range of ecosystems (fig. S1), oVOCs deposited at high

rates. The sum of methyl vinyl ketone (MVK) and methacrolein (MAC), which account for about 80% of the carbon in the initial stage of isoprene oxidation, exhibits the fastest deposition rates. R_c for deciduous ecosystems is much smaller than predicted and falls along a trend that would be expected for O_3 ($f_0 = 1$) (Fig. 1). This suggests that oVOCs, like O_3 , are immediately lost once they enter a leaf through stomata. Tropical ecosystems exhibit the fastest deposition rates (R_s are 2.6 to 3.5 times smaller than predicted), and the observed deposition velocities for MVK+MAC are as large as for O_3 (up to 2.4 cm/s). We measured similarly high-deposition fluxes for other oVOCs, including acetaldehyde, MVK+MAC, hydroxyacetone, glycolaldehyde, C_5 carbonyls, and nopinone (Fig. 2). All oVOCs deposited on the vegetation except acetaldehyde, which exhibited net emissions during a hot period at the beginning of the study because it is also produced by vegetation (11). Because of the bidirectional exchange of acetaldehyde, v_d should be regarded as a net deposition velocity. All oVOCs investigated here form during the photochemical oxidation of isoprene and certain monoterpenes. The vertical profiles suggest that deposition mainly occurs via uptake by vegetation elements, with the upper 70% of the canopy (Fig. 2) accounting for more than ~97% of the total de-

position flux for all measured oVOCs. After correction for different molecular diffusivities in air, the corresponding R_c s of oVOCs (except acetaldehyde) fall within 25% of O_3 . Because of the bidirectional exchange, R_c for acetaldehyde is 60% higher than that of O_3 (Fig. 2). Large deposition fluxes imply that the internal concentration (C_i) of these oVOCs is small compared with the ambient concentration (C_a).

To investigate mechanisms that can potentially influence the uptake of oVOCs—in particular, MVK and MAC—we performed a series of leaf cuvette experiments with *Populus trichocarpa* $\times$ *deltoides*. We observed a linear flux-concentration relationship between the uptake (the flux) of oVOCs by a leaf as a function of oVOC concentration (figs. S3 and S5), which is indicative of enzymatic reactions metabolizing oVOCs. The compensation point (C_p) of a compound, defined as the concentration at which the net uptake is zero, typically followed an exponential increase with leaf temperature (fig. S6). When oVOCs form or decompose inside a leaf, the C_p is typically greater than zero, resulting in emission below and uptake above the C_p . The mesophyll resistance (R_m), expressed as a deposition velocity ($v_m = 1/R_m$), was highest between 15° and 20°C, dropping substantially above a narrow temperature range between 25° and 28°C (fig. S6). The light response curves of v_m exhibited a functional form similar to electron transport.

Plants possess the ability to detoxify through various mechanisms [via oxidative stress or conversion by the aldehyde dehydrogenase (ALDH) family]. As an example, ALDHs are a protein superfamily of nicotinamide adenine dinucleotide phosphate (NADP⁺)-dependent enzymes known to oxidize a wide range of carbonyls. ALDH enzymes play an important role in the detoxification of aldehydes by oxidizing these to their corresponding carboxylic acids (12). Stress-induced up-regulation of these and potentially other enzymes (such as various peroxidases) could help reduce concentrations of aldehydes and other oVOCs that would otherwise build up to toxic levels.

On the basis of exposure experiments with *P. trichocarpa* $\times$ *deltoides*, we suggest that chemical and mechanical stress affects uptake rates of acetaldehyde and MVK (Fig. 3). For both plants, v_m for acetaldehyde dropped during fumigation with MVK but changed little for MVK. The corresponding C_p for acetaldehyde increased during the fumigation period, suggesting increased internal production occurring as a response to oxidative or metabolic stress. During MVK fumigation, conversion rates of aldehydes may have also decreased through enzyme saturation or depletion of reactive oxygen species (ROSS), which would explain the relatively small change of v_m for MVK during fumigation as opposed to a large increase immediately after fumigation. For the MVK experiment, the post-exposure v_m drastically increased for acetaldehyde (approximately twofold) and MVK (~20-fold), suggesting short-term up-regulation of metabolic activity in response to MVK exposure during the previous days; however, v_m decreased

Fig. 1. Canopy resistance (R_c) for MVK+MAC plotted versus leaf area index (LAI) for six field sites. The black solid line represents dry deposition, where the reactivity factor f_0 was set to 1 (maximum deposition). The dotted line shows R_c with a dry deposition that would be conventionally used ($f_0 = 0$). LAI data are plotted as mean $\pm$ SD ($n = 10$ LAI measurements); the SD for R_c was calculated as the sum of systematic and random errors associated with turbulent flux measurements (14) and the errors associated with instrument precision for wind measurements through use of a sonic anemometer.

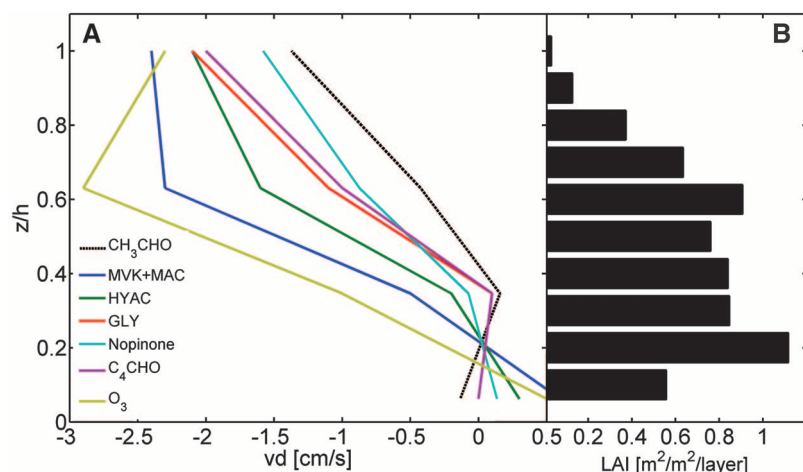
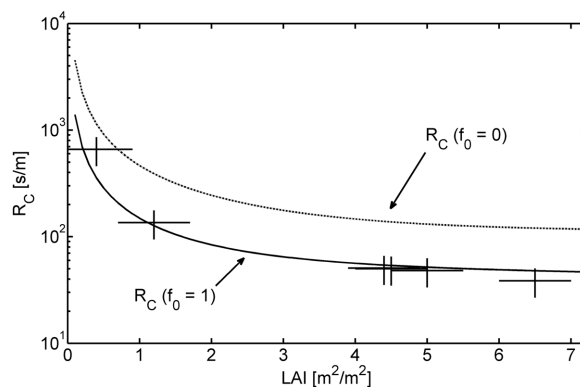


Fig. 2. (A) Canopy-integrated deposition velocity for ozone (O_3), C_5 carbonyls (C_4CHO), nopinone, glycolaldehyde (GLY), hydroxyacetone (HYAC), MVK+MAC, and acetaldehyde (CH_3CHO) as a function of normalized canopy height (z/h), where a z/h of 1 represents the top of the canopy. (B) The LAI per layer.

after one day in both cases. The v_m for MVK increasing direct or indirect enzymatic reactions, rather than purely nonenzymatic reactions (such as Michael addition), probably regulated the metabolic consumption. MVK fumigation also resulted in physiological changes. After a certain exposure period, stomatal conductance (G_s) and photosynthesis dropped by 30 to 50% and 20 to 50%, respectively, each day. G_s and photosynthesis, however, recovered every morning. This corroborates the finding that MVK actively goes through stomata, inducing a biological response in the mesophyll.

We did not observe any physiological changes (G_s and photosynthesis) for plant B during the course of the O_3 fumigation experiment. v_m for MVK increased by ~70% immediately after we exposed plant B to O_3 and gradually increased by

another ~25% during the course of the fumigation, where it remained after we turned off the O_3 supply (Fig. 3). We observed similar behavior for acetaldehyde. For both compounds, v_m remained high for 2 days after stopping the fumigation. A set of experiments inducing mechanical wounding resulted in increased uptake of MVK, which is qualitatively similar to the chemical exposure experiments. The uptake of acetaldehyde decreased and occurred in parallel with an increasing C_p . This is consistent with the idea that mechanical wounding increases the internal production of acetaldehyde (11). Upon recovery, the uptake rates (v_m) for acetaldehyde increased subsequently and at times exceeded the pre-wounding values.

The increase of v_m for acetaldehyde and MVK after (plant A and B) and during (plant B) fumigation imply that these plants responded to acute

chemical exposure by up-regulating the activity of processes responsible for detoxification. Quantitative polymerase chain reaction identified changes in several characteristic genes indicative of enhanced metabolic activity as a response to chemical and mechanical stress (table S3). All chemical treatments (MVK and O_3) showed elevated gene expression patterns related to ROS (superoxide dismutase and ascorbate peroxidase). Mechanical wounding and chemical exposure experiments (MVK and O_3) resulted in up-regulation of aldehyde oxidase (AAO2) and aldehyde dehydrogenase (ALDH2). We observed elevated levels of p450 cytochrome, which is typically associated with the oxidation of organic substances, for all chemical treatments along with increases in WRKY transcription factors, encoding proteins involved in plant responses to biotic and abiotic stresses. These changes in gene-expression patterns support the gas-exchange measurements, showing that plants can adjust their metabolism (meaning, their v_m) and increase oVOC decomposition as a response to environmental factors.

The presented laboratory and field observations show that oVOCs can be efficiently metabolized by plants through constitutive and induced detoxification mechanisms. Because the general route of atmospheric photooxidation of NMVOCs goes through the formation of carbonyls and hydroxycarbonyls, these findings have consequences for understanding the atmospheric evolution of these oVOCs. To place these findings on a broader scale, we modified the dry deposition scheme for oVOCs in a comprehensive global chemistry and transport model (13, 14). Fast metabolic conversion of oVOCs was incorporated, according to our field observations, by setting f_0 to 1 (9). The total dry deposition flux of organics on a carbon basis increases (by up to 110%) relative to previous estimates (Fig. 4). Large changes especially occur in tropical regions such as the Amazon, where the annual dry deposition flux increases between 65 and 85%. Globally, dry deposition of organics increases by about 36%. More importantly, deposition fluxes are substantially altered because of increases (50 to 100%) in dry deposition of relatively insoluble species [for example, carbonyls with Henry's Law Constant (HLC) of <50 M/atm] leading to decreases (up to 30%) in wet deposition of certain soluble species [for example, peroxides with HLCs of >300 M/atm (fig. S8)]. Higher dry deposition of relatively insoluble species also leads to a decrease in dry deposition of certain peroxides because of lower atmospheric concentrations in the gas phase. Modeled oVOC concentrations in the surface layer change substantially (30 to 60%). These results have consequences for capturing the dynamic behavior and repartitioning between NMVOC oxidation products and SOA (5, 14, 15). The modifications change OH radical concentrations by up to 15% above the land surface layer (fig. S11). Tropospheric O_3 concentrations are slightly reduced (by 1 to 3%) in the Northern Hemisphere and enhanced (by 0.5 to 1.5%) in the tropical land regions (fig. S10).

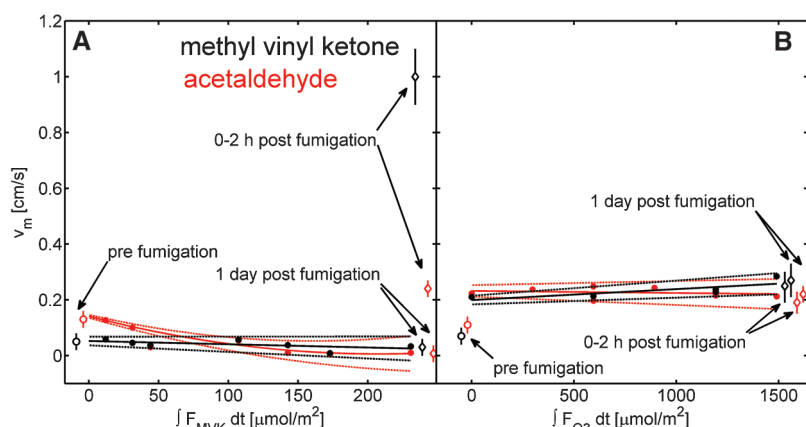


Fig. 3. Mesophyllic deposition velocity ($v_m = 1/R_m$) for acetaldehyde and MVK; v_m is plotted as a function of cumulative (A) MVK and (B) O_3 uptake. Prefumigation levels of v_m (open circles) are inserted at the beginning; postfumigation levels of v_m (open diamonds) are inserted at the end. 95% confidence intervals (CIs) [$n = 6$ samples (O_3 fumigation); $n = 7$ samples (MVK fumigation)] are shown as vertical bars for pre- and postfumigation experiments; the dotted lines represent the 95% CI during the fumigation experiment obtained from a polynomial regression (second order) depicted by the solid lines.

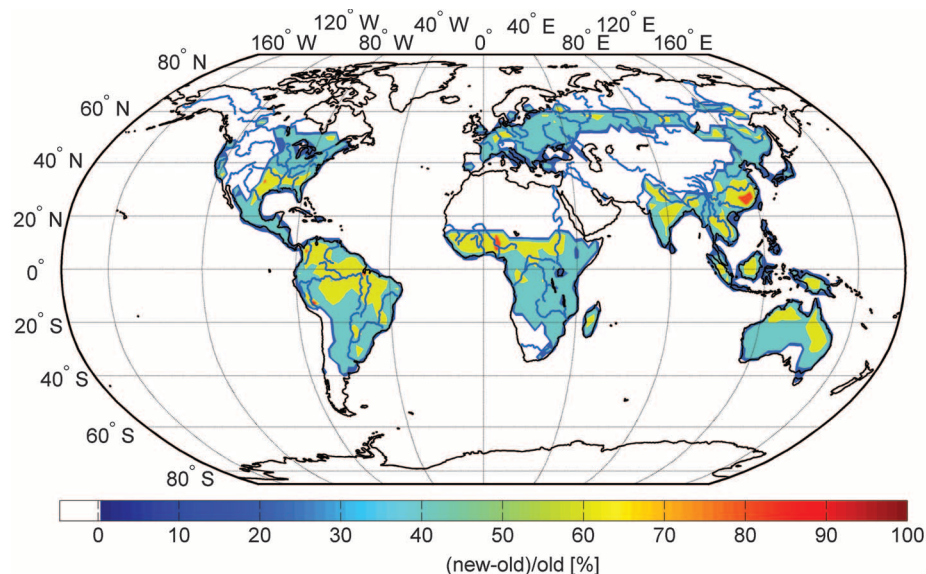


Fig. 4. Relative change of the total annual dry deposition flux of oVOC on the basis of a modified dry deposition scheme in which oVOCs are assumed to be rapidly metabolized within the leaf.

Dry deposition of organic trace gases addresses a poorly quantified process in the atmosphere (3, 10). We estimate a lower and upper bound for the annual deposition flux of gas phase oVOCs between 37 and 56% relative to the annual NMVOC emission flux on a carbon basis (table S4). It is conceivable that oVOC deposition fluxes to vegetation could increase as a consequence of acute or chronic exposure to high O₃ concentrations in polluted regions (16).

References and Notes

1. A. H. Goldstein, I. Galbally, *Environ. Sci. Technol.* **41**, 1515 (2007).
2. A. Guenther *et al.*, *Atmos. Chem. Phys.* **6**, 3181 (2006).
3. M. Hallquist *et al.*, *Atmos. Chem. Phys.* **9**, 5155 (2009).
4. R. Atkinson, J. Arey, *Chem. Rev.* **103**, 4605 (2003).
5. F. Paulot *et al.*, *Science* **325**, 730 (2009).
6. J. Lelieveld *et al.*, *Nature* **452**, 737 (2008).
7. W. J. Collins, R. G. Derwent, C. E. Johnson, D. S. Stevenson, *Clim. Change* **52**, 453 (2004).
8. C. A. Paulson *et al.*, *J. Appl. Meteorol.* **9**, 857 (1970).
9. M. L. Wesely, *Atmos. Environ.* **23**, 1293 (1989).
10. L. Zhang *et al.*, *Atmos. Environ.* **36**, 537 (2002).
11. R. Fall, *Chem. Rev.* **103**, 4941 (2003).
12. H. H. Kirch, D. Bartels, Y. Wei, P. S. Schnable, A. J. Wood, *Trends Plant Sci.* **9**, 371 (2004).
13. L. K. Emmons *et al.*, *Geosci. Model Dev.* **3**, 43 (2010).
14. Materials and methods are available as supporting material on Science Online
15. J. L. Jimenez *et al.*, *Science* **326**, 1525 (2010).
16. K. L. Denman *et al.*, in IPCC 4th Assessment Report, S. Solomon *et al.*, Eds. (Cambridge Univ. Press, Cambridge, 2007), chap. 7.
17. The National Center for Atmospheric Research is operated by the University Corporation for Atmospheric Research under sponsorship from the National Science Foundation.

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Transient Middle Eocene Atmospheric CO₂ and Temperature Variations

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The long-term warmth of the Eocene (~56 to 34 million years ago) is commonly associated with elevated partial pressure of atmospheric carbon dioxide ($p\text{CO}_2$). However, a direct relationship between the two has not been established for short-term climate perturbations. We reconstructed changes in both $p\text{CO}_2$ and temperature over an episode of transient global warming called the Middle Eocene Climatic Optimum (MECO; ~40 million years ago). Organic molecular paleothermometry indicates a warming of southwest Pacific sea surface temperatures (SSTs) by 3° to 6°C. Reconstructions of $p\text{CO}_2$ indicate a concomitant increase by a factor of 2 to 3. The marked consistency between SST and $p\text{CO}_2$ trends during the MECO suggests that elevated $p\text{CO}_2$ played a major role in global warming during the MECO.

The Middle Eocene Climatic Optimum [MECO; ~40 million years ago (Ma)] (1) interrupts a long-term middle Eocene cooling trend (2), with a globally uniform 4° to 6°C warming of both surface and deep oceans within ~400,000 years, as derived from foraminiferal stable oxygen isotope records (3). A decrease in carbonate mass accumulation rates during the MECO argues for ocean acidification induced by a rise in $p\text{CO}_2$ (3). Application of paleo- $p\text{CO}_2$ proxies across the MECO has yet to confirm whether $p\text{CO}_2$ changes are indeed associated with this interval of transient warming.

We investigated a sedimentary succession spanning the MECO recovered from the East Tasman Plateau at Ocean Drilling Program (ODP) Site 1172, which at that time was situated on the shelf (~65°S paleolatitude; Fig. 1 and figs. S1 and S2) (4, 5). To fully capture the magnitude of the sea surface temperature (SST) change associated

with the MECO at this site, we applied two independent temperature proxies: the alkenone unsaturation index (U_{37}^K) (6) and the index of tetraethers consisting of 86 carbon atoms (TEX_{86}) (5, 7) (fig. S3). At the onset of the MECO, U_{37}^K and TEX_{86} indicate a rise in SST of 3°C and 6°C, respectively, which, also at this location, stands out as an interruption of long-term middle Eocene cooling (Fig. 2). Bulk carbonate oxygen isotope values ($\delta^{18}\text{O}$) decrease by 1.0 to 1.2 per mil (‰), which, if controlled by SST only, also indicate a SST rise of ~4° to 5°C (5).

Additional evidence of warming is derived from assemblages of hypnozyotic organic cysts of surface-dwelling dinoflagellates (dinocysts) (5). Whereas the middle Eocene dinocyst record at ODP Site 1172 is dominated by taxa that are endemic to the Southern Ocean (8), an incursion of low-latitude dinocyst taxa characterizes the MECO (Fig. 2 and fig. S4). A SST increase of 3° to 6°C is consistent with inferences from benthic foraminiferal and fine-fraction carbonate oxygen isotope records at other sites (1, 3). The U_{37}^K and TEX_{86} proxies are independent of seawater $\delta^{18}\text{O}$. Hence, the consistent magnitude of warming between the proxies suggests that the carbonate $\delta^{18}\text{O}$ records were not affected by a change in $\delta^{18}\text{O}$ of seawater, and that global ice volume did not change considerably during the MECO.

Absolute SSTs as indicated by U_{37}^K and TEX_{86} are consistent, with 26°C or 24°C just below the onset of the MECO for the two proxies, respective-

ly, and peak MECO SSTs exceeding 28°C. These SSTs are much (~10°C) higher than those derived from fine-fraction carbonate oxygen isotope measurements from elsewhere in the Southern Ocean (1, 3). At least part of this large discrepancy is most likely the result of diagenetic alteration of calcite (9).

We assessed $p\text{CO}_2$ changes by determining the stable carbon isotopic composition ($\delta^{13}\text{C}$) of alkenones, long-chained ketones exclusively synthesized by specific haptophyte algae. Carbon isotopic fractionation during carbon fixation (ϵ_p) by haptophyte algae varies as a function of dissolved CO₂ [$\text{CO}_{2(\text{aq})}$] (10, 11), specific cell physiological parameters (which show good correspondence to the surface-water concentrations of soluble phosphate), and other environmental parameters, primarily light intensity (5). The carbon isotopic composition of diunsaturated alkenones ($\delta^{13}\text{C}_{\text{C}_{37:2}}$) ranges between -32.5 and -35.5‰ (fig. S3). We used bulk carbonate $\delta^{13}\text{C}$ to estimate the $\delta^{13}\text{C}$ value of the dissolved inorganic carbon (DIC) pool in seawater (5) to determine ϵ_p . The data show background ϵ_p values of 21 to 22‰ rising up to 24.5‰ during MECO (Figs. 2 and 3 and fig. S3). The relationship between ϵ_p and $p\text{CO}_2$ is exponential, which results in a relatively large uncertainty in reconstructed $p\text{CO}_2$ levels with high ϵ_p values (Fig. 3). Temperature variations, however, play a minor role in the range of temperatures indicated by TEX_{86} and U_{37}^K (Fig. 2) and cannot explain the high ϵ_p values (Fig. 3). It seems unlikely that changes in light intensity (12) influenced ϵ_p substantially at ODP Site 1172 (5). The soluble phosphate concentration exerts a strong influence on the relation between ϵ_p and $p\text{CO}_2$, particularly if ϵ_p values are high (5).

To evaluate all possible absolute $p\text{CO}_2$ estimates from our record, we applied the full range of present-day surface-water phosphate concentrations. These vary between 0 $\mu\text{mol liter}^{-1}$ in the oligotrophic gyres to >2 $\mu\text{mol liter}^{-1}$ in the Southern Ocean (5) (fig. S5). Yet even when phosphate concentrations of 0 $\mu\text{mol liter}^{-1}$ are assumed, ϵ_p values between 21.2‰ and 24.5‰ yield $p\text{CO}_2$ estimates between 600 parts per million by volume (ppmv) before the MECO and 6400 ppmv during the MECO (Figs. 2 and 3). Hence, elevated levels of $p\text{CO}_2$ must in part be responsible for the high ϵ_p values, with middle Eocene $p\text{CO}_2$

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being more than twice the pre-industrial value. When we assume maximal phosphate concentrations of $2 \mu\text{mol liter}^{-1}$, $p\text{CO}_2$ ranges between

~ 2500 and $\sim 24,000$ ppmv (Figs. 2 and 3). The marginal marine Eocene East Tasman Plateau (4) likely experienced phosphate concentrations that

were higher than $0 \mu\text{mol liter}^{-1}$ but lower than $2 \mu\text{mol liter}^{-1}$, because closed oceanic gateways during the Eocene (13) prevented mixing associated with the Antarctic Circumpolar Current (ACC) that causes high phosphate levels in the present-day Southern Ocean. Eocene southwest Pacific surface-water phosphate concentrations were unlikely to have exceeded $1 \mu\text{mol liter}^{-1}$, which implies maximum $p\text{CO}_2$ estimates of 1600 ppmv just before the MECO and 15,000 ppmv during the MECO (Figs. 2 and 3). With a realistic range of phosphate concentrations, $p\text{CO}_2$ values were between 600 and 1600 ppmv just before the MECO, which is in line with previous estimates of middle Eocene $p\text{CO}_2$ values using the same proxy (14), and rose to between 6400 and 15,000 ppmv during the MECO (Fig. 2, light gray band). MECO values exceed any previous Eocene alkenone-based estimate even when we assume phosphate concentrations of $0 \mu\text{mol liter}^{-1}$ (5). Despite uncertainties regarding absolute $p\text{CO}_2$ values, we note that the trends in $p\text{CO}_2$ follow those in the SST records remarkably well (Fig. 2).

Surface-water phosphate concentrations, however, may have varied in a marginal marine setting at ODP Site 1172. A tool for the reconstruction of phosphate concentration uses the remains of dinoflagellates (dinocysts), which are known to be

Fig. 1. Paleogeographic configuration of the southern high latitudes during the middle Eocene (~ 49 to 37 Ma; map was obtained from www.ods.de) and ocean surface current configurations inferred from general circulation model experiments (13). The orange star indicates the paleogeographic location of ODP Site 1172 at 65°S in the southwest Pacific Ocean (24), under the influence of the Antarctic-derived Tasman Current (TC).

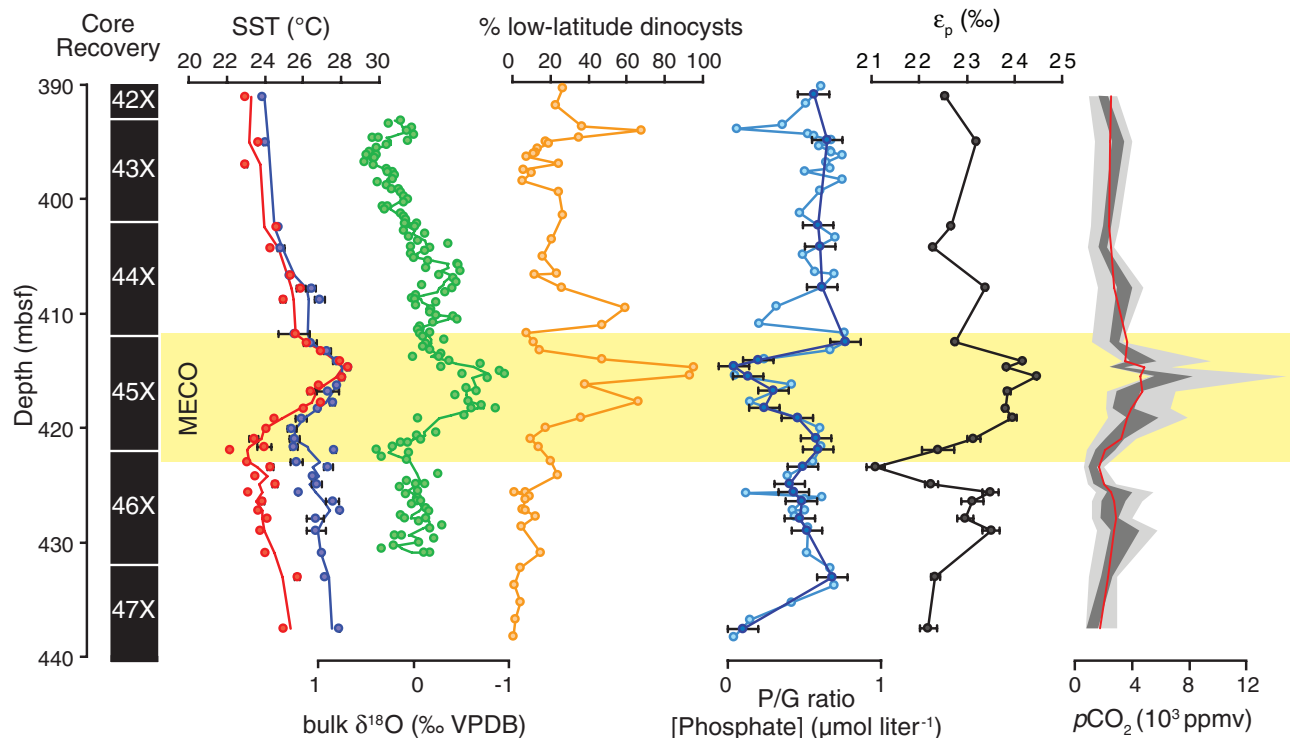


Fig. 2. Geochemical and palynological results across the MECO at ODP Site 1172, Hole A, cores 42X to 47X. The MECO is identified by integrating magnetostratigraphy (25), biostratigraphy (25), and chemostratigraphy (3) (see fig. S2). The U_{37}^K (purple), TEX_{86} (red), and bulk $\delta^{18}\text{O}$ (green) SST reconstructions show a warming of 3° to 6°C . The yellow shaded area delimits the MECO interval. The increase in percentage of low-latitude dinocysts (in orange) at the expense of endemic dinocysts during the MECO illustrates biotic response to warming. We estimated $p\text{CO}_2$ from carbon isotopic fractionation during carbon fixation (ϵ_p ; black) by haptophyte algae with phosphate

concentrations between 0 and $1 \mu\text{mol liter}^{-1}$ (light gray band). We further constrained phosphate estimates (dark blue line) by allowing phosphate concentrations to vary between $0.1 \pm 0.1 \mu\text{mol liter}^{-1}$ and $0.9 \pm 0.1 \mu\text{mol liter}^{-1}$ as a function of the ratio of peridinioid over gonyaulacoid dinocysts (P/G ratio; light blue line). This results in further constrained $p\text{CO}_2$ estimates (dark gray band). TEX_{86} , U_{37}^K , oxygen isotope, and $p\text{CO}_2$ data are plotted with a 3-point running mean (solid orange, purple, green, and red lines, respectively). The error bars on TEX_{86} and U_{37}^K represent analytical error. On ϵ_p the error bars represent the difference between the use of TEX_{86} and U_{37}^K to determine ϵ_p .

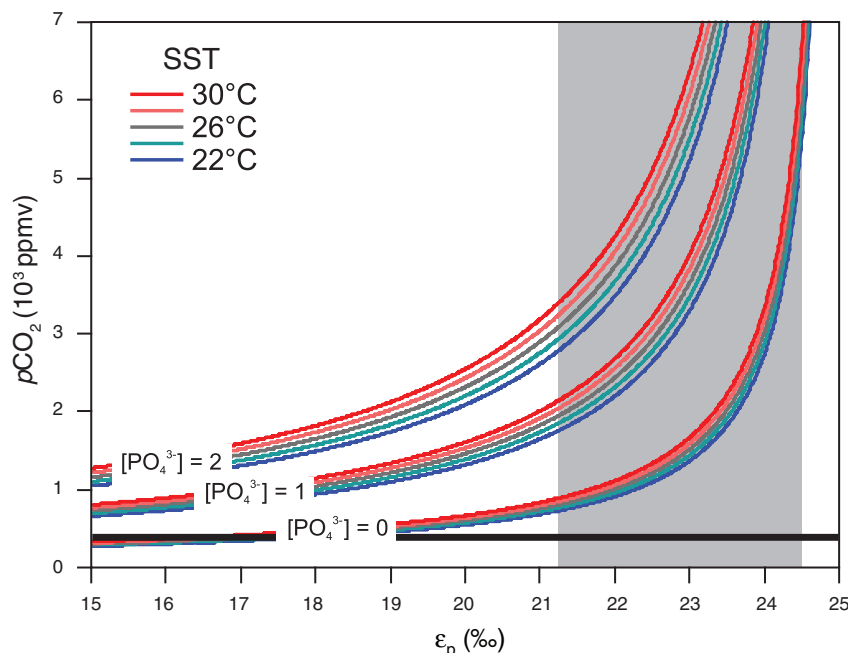


Fig. 3. Relationship between ϵ_p [versus VPDB (Vienna pee dee belemnite) standard] and $p\text{CO}_2$. The phosphate concentration ranges plotted are those from the present-day surface ocean, and the SST ranges (22° to 30°C) are those inferred from TEX_{86} and U_{37}^K data presented in Fig. 2 and a suite of Southern Ocean sites (22, 26–28). The gray vertical bar indicates the values for ϵ_p as reconstructed for the MECO interval at ODP Site 1172, with use of TEX_{86} -based and U_{37}^K -based SST reconstructions for these same levels, and bulk carbonate $\delta^{13}\text{C}$ measurements on the same section. Black horizontal line represents present-day $p\text{CO}_2$. Figure is modified from Pagani *et al.* (10).

extremely sensitive to surface-water nutrient availability changes (5). The ratio between peridinioid and gonyaulacoid dinocyst groups (the P/G ratio) is often used to reconstruct changes in relative nutrient abundance (15). The MECO at ODP Site 1172 shows a major decrease in the P/G ratio (16) (Fig. 2), suggesting a decrease in nutrient concentrations (16). As an experiment, we allowed phosphate to linearly vary as a function of the P/G ratio (Fig. 2) (5). The most prominent shift toward low P/G ratios occurs at MECO warming, resulting in lower phosphate concentration estimates. Also, when this drop in phosphate is taken into account, $p\text{CO}_2$ rises during the MECO and follows the SST trends (Fig. 2, dark gray band). Hence, regardless of the constraints on phosphate concentrations and other environmental parameters, $p\text{CO}_2$ levels must have been substantially higher during the MECO relative to the middle Eocene background.

One outstanding issue is the source of carbon responsible for the increase in middle Eocene atmospheric CO_2 . The rise in $p\text{CO}_2$ by 2000 to 3000 ppmv emerging from our data requires a carbon source capable of injecting vast amounts of carbon into the atmosphere. Moreover, the absence of a prominent negative carbon isotope excursion excludes reservoirs with $\delta^{13}\text{C}$ signatures below that of marine DIC (3). One mechanism capable of emanating carbon with such a geochemical signature is the metamorphic alteration of carbonates (decarbonation) (1). Massive decarbonation occurred until the late Eocene, with the subduction of vast amounts of Tethyan Ocean pelagic carbonates

under Asia as India drifted northward (17–19). However, the flux of carbon required to increase $p\text{CO}_2$ by 2000 to 3000 ppmv within ~400,000 years appears too high to invoke metamorphic (volcanic) outgassing as the sole mechanism.

Our $p\text{CO}_2$ and SST reconstructions allow for a tentative assessment of high-latitude climate sensitivity to CO_2 forcing on ~100,000-year time scales, assuming that all MECO warming was caused by $p\text{CO}_2$ and associated feedbacks. With an average 5°C SST increase and a factor of 2 to 3 increase in $p\text{CO}_2$, we arrive at a climate sensitivity of ~ 2° to 5°C per $p\text{CO}_2$ doubling. When we consider the $p\text{CO}_2$ decline from the Middle Eocene (~2000 ppmv; this study) to the latest Eocene (~1000 ppmv) (20) and the coeval high-latitude temperature decline (~ 3.5°C) (21, 22), we derive similar values. Thus, long-term climate sensitivity to $p\text{CO}_2$ forcing in a world without the amplifying effects of ice-albedo feedbacks (23) may have been larger than previously anticipated.

References and Notes

1. S. M. Bohaty, J. C. Zachos, *Geology* **31**, 1017 (2003).
2. J. Zachos, M. Pagani, L. Sloan, E. Thomas, K. Billups, *Science* **292**, 686 (2001).
3. S. M. Bohaty, J. C. Zachos, F. Florindo, M. L. Delaney, *Paleoceanography* **24**, PA2207 (2009).
4. N. F. Exon, J. P. Kennett, M. J. Malone, Eds., *Proceedings of the Ocean Drilling Program, Scientific Results* (U.S. Government Printing Office, College Station, TX, 2003), vol. 189.
5. See supporting material on Science Online.

6. S. C. Brassell, G. Eglinton, I. T. Marlowe, U. Pflaumann, M. Sarnthein, *Nature* **320**, 129 (1986).
7. S. Schouten, E. C. Hopmans, E. Schefuß, J. S. Sinninghe Damsté, *Earth Planet. Sci. Lett.* **204**, 265 (2002).
8. H. Brinkhuis, S. Sengers, A. Sluijs, J. Warnaar, G. L. Williams, in *Proceedings of the Ocean Drilling Program, Scientific Results*, N. F. Exon, J. P. Kennett, M. J. Malone, Eds. (U.S. Government Printing Office, College Station, TX, 2003), vol. 189, pp. 1–48.
9. P. N. Pearson *et al.*, *Nature* **413**, 481 (2001).
10. M. Pagani, *Philos. Trans. R. Soc. London Ser. A* **360**, 609 (2002).
11. Surface ocean $\text{CO}_2(\text{aq})$ originates from atmospheric CO_2 and deep waters, of which the latter is of major importance in marginal marine upwelling areas. Changes in upwelling through time may substantially change $\text{CO}_2(\text{aq})$ and hence would skew the reconstructed $p\text{CO}_2$ record. At Site 1172 we argue that a change in upwelling is not responsible for the signal we recorded in the alkenones, because that would have led to prominent shifts in the bulk carbonate carbon isotope profile (figs. S2 and S3).
12. B. Rost, I. Zondervan, U. Riebesell, *Limnol. Oceanogr.* **47**, 120 (2002).
13. M. Huber *et al.*, *Paleoceanography* **19**, PA4026 (2004).
14. M. Pagani, J. C. Zachos, K. H. Freeman, B. Tipler, S. Bohaty, *Science* **309**, 600 (2005); 10.1126/science.1110063.
15. A. Sluijs, J. Pross, H. Brinkhuis, *Earth Sci. Rev.* **68**, 281 (2005).
16. U. Röhl *et al.*, *Geophys. Monogr.* **151**, 127 (2004).
17. D. V. Kent, G. Muttoni, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 16065 (2008).
18. J. C. Aitchison, J. R. Ali, A. M. Davis, *J. Geophys. Res.* **112**, B05423 (2007).
19. G. Dupont-Nivet, C. Hoorn, M. Konert, *Geology* **36**, 987 (2008).
20. P. N. Pearson, G. L. Foster, B. S. Wade, *Nature* **461**, 1110 (2009).
21. J. C. Zachos, G. R. Dickens, R. E. Zeebe, *Nature* **451**, 279 (2008).
22. Z. Liu *et al.*, *Science* **323**, 1187 (2009).
23. M. Pagani, Z. Liu, J. LaRiviere, A. C. Ravelo, *Nat. Geosci.* **3**, 27 (2010).
24. N. Exon, J. P. Kennett, M. Malone, *Geophys. Monogr.* **151**, 367 (2004).
25. C. E. Stickley *et al.*, in *Proceedings of the Ocean Drilling Program, Scientific Results*, N. F. Exon, J. P. Kennett, M. J. Malone, Eds. (U.S. Government Printing Office, College Station, TX, 2003), vol. 189, pp. 1–57.
26. C. E. Burgess *et al.*, *Geology* **36**, 651 (2008).
27. C. J. Hollis *et al.*, *Geology* **37**, 99 (2009).
28. P. K. Bijl *et al.*, *Nature* **461**, 776 (2009).
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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S5

Tables S1 to S3

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Intestinal Stem Cell Replacement Follows a Pattern of Neutral Drift

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With the capacity for rapid self-renewal and regeneration, the intestinal epithelium is stereotypical of stem cell-supported tissues. Yet the pattern of stem cell turnover remains in question. Applying analytical methods from population dynamics and statistical physics to an inducible genetic labeling system, we showed that clone size distributions conform to a distinctive scaling behavior at short times. This result demonstrates that intestinal stem cells form an equipotent population in which the loss of a stem cell is compensated by the multiplication of a neighbor, leading to neutral drift dynamics in which clones expand and contract at random until they either take over the crypt or they are lost. Combined with long-term clonal fate data, we show that the rate of stem cell replacement is comparable to the cell division rate, implying that neutral drift and symmetrical cell divisions are central to stem cell homeostasis.

Theories of epithelial cell renewal place stem cells at the apex of proliferative hierarchies, possessing the lifetime property of self-renewal (1). In homeostasis, the number of stem cells remains fixed, imposing an absolute requirement for fate asymmetry in the daughters of dividing stem cells, such that only half are retained as stem cells. Fate asymmetry can be achieved either by being the invariant result of every division (intrinsic asymmetry) or by being orchestrated from the whole population, where cell fate following stem cell division is specified only up to some probability (population asymmetry) (1, 2). These alternative models suggest very different mechanisms of fate regulation, yet their identification in normal tissues remains elusive.

In the intestinal crypt, the apex of the proliferative hierarchy is associated with the anchored cells of the crypt base (3). Heterogeneity in expression of fate-determining genes and in cell cycle characteristics has suggested that at least two populations (Lgr5⁺ and Bmi1⁺) of crypt stem cells may exist (4, 5). For neither population has the pattern of self-renewal been revealed.

Recent studies have provided evidence in support of intrinsic asymmetry by showing that crypt base cells are more likely to show an oriented spindle than cells higher in the crypt, and this correlates with asymmetric DNA segregation during division (6, 7). However, the phenomenon of monoclonal conversion, whereby crypts become monophenotypic (clonal) with time after genetic marking of individual cells (8–10), presents an apparent experimental paradox. This observation rules out truly invariant modes of cell division involving multiple stem cells and has been explained by rare errors in intrinsic asymmetry (11–13). Hence, the consensus view remains that intestinal stem cells divide asymmetrically due

either to inherent properties or environmental cues (3). Here we show that the dynamics of clonal growth leading to monoclonal conversion is directly related to the pattern of steady-state self-renewal.

Intestinal clones were induced by low-level Cre-mediated recombination in *Ahcre^{ERT}* animals (targeting potentially all the proliferative populations within the crypt with a single treatment) crossed to Cre-reporter strains as described previously, and were visualized in tissue whole mounts or sections [Fig. 1, A to J, and supporting online material (SOM) S-I]. With only 1.9 ± 0.7% of crypts containing labeled cells at 2 weeks after induction, the vast majority of clones were expected to derive from single cells. By imaging the crypt base, we first determined the proportion that have achieved monoclonal conversion (Fig. 1, I and J). Crypts can become monoclonal a short time after induction, such that ~50% are fully labeled within 8 weeks (Fig. 1K). The dynamics of monoclonal conversion can also be tracked by scoring the number of differentiated progeny that emerge from the crypts (Fig. 1, L to N, and fig. S1). After pulse labeling, cells exported from crypts form one to three clear migration streams on villi (Fig. 1, B and C). On the villus, clones comprised both Goblet and absorptive cell lineages, and within the crypt, clones contained Paneth cells. Each fully labeled crypt supports a clone width of $w_{\max} = 6.8 \pm 1.3$ cells (mean ± SD, $n = 155$) along the villus. As with the crypt base analysis, we found that 50% of clones were of full width (i.e., six cells or more) within 8 weeks, corresponding to 50% monoclonal crypts at this time point (Fig. 1M).

Monoclonal conversion rules out a simple model of tissue maintenance that originates from a population of long-lived stem cells following a strict pattern of asymmetric division, and can be explained by two classes of behavior. First, crypts could be maintained by a hierarchy in which a single stem cell generates, through a sequence of asymmetric divisions, stem cells with a more limited proliferative potential (14) (Fig. 2A). Second, tissue could be maintained by an equipotent stem cell population, in which stem cell loss is perfectly compensated by the multiplication of others (15–17) (Fig. 2B). Any detailed model of self-

renewal will belong to one of these two classes (SOM S-II).

Although both behaviors predict attrition of surviving clones and drift toward monoclonality, the full range of clonal fate data allows us to discriminate between them. To understand how, we draw upon concepts from statistical physics applied to population dynamics. Within an equipotent cell population, ongoing replacement leads to “neutral drift” of the clonal population (17). In this case, if the stem cell pool were not limited by anatomical constraints, clonal evolution would settle into a characteristic behavior in which the size distribution acquires a “scaling form”: defining $P_n(t)$ as the fraction of surviving clones that host n stem cells at a time t after induction (see SOM S-III) (17, 18)

$$P_n(t) = \frac{1}{\langle n(t) \rangle} F\left(\frac{n}{\langle n(t) \rangle}\right) \quad (1)$$

where $\langle n(t) \rangle$ denotes the average number of stem cells in a surviving clone. The scaling function, $F(x)$, is “universal,” dependent only on the spatial organization of stem cells. From Eq. 1, it follows that, if $\langle n(t) \rangle P_n(t)$ is plotted against $n/\langle n(t) \rangle$, the size distributions will follow the same curve irrespective of the time t . In the crypt, where the stem cell compartment is limited, scaling is transient and would eventually fail when a noticeable fraction of crypts become monoclonal.

By contrast, if replacement occurs hierarchically, then clones derived from the “master” stem cell will increase steadily in size, whereas those derived from its shorter-lived progeny will exhibit limited growth followed by loss. Crucially, the mixture of these two behaviors leads to binomial size distributions that do not scale (SOM S-V).

Clone size is impossible to measure in absolute terms in the intestine because of the constant migration and loss of cells. However, we can access $P_n(t)$ indirectly: The cohesion of clones within the crypt, and the proportionality of the clone width emerging from the crypt to the villus (SOM S-I), show that clonal expansion is tied to the circumference at the crypt base. Therefore, the clone width, relative to that of fully labeled crypts, serves as a proxy for the fraction of labeled stem cells within the crypt. Formally, a clone of width w on the villus is associated with a clone covering a fraction $f(w) = w/w_{\max}$ of the circumference at the crypt base. The number of stem cells associated with a clone of width w is given by $n = f(w)N_{\text{stem}}$, where N_{stem} denotes the number of stem cells surrounding the crypt base. With this assignment, we find that for $t \leq 4$ weeks, where the vast majority of crypts have yet to become monoclonal, the size distributions show scaling behavior (Fig. 2D), an unambiguous signature of neutral drift dynamics.

The growth rate, $\langle n(t) \rangle$, and the form of $F(x)$ have the potential to offer further insight into the pattern of stem cell fate. In particular, if stem cells are organized into a one-dimensional arrangement, with cell replacement effected by neighboring stem cells (Fig. 3, A and B), then the average size of surviving clones grows as a square root of time,

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$\langle n(t) \rangle \approx \sqrt{\lambda t}$, with λ the stem cell replacement rate, and the scaling function is predicted to take the form (SOM S-III)

$$F(x) = (\pi x/2) \exp[-\pi x^2/4] \quad (2)$$

Referring to the experimental data, the coincidence of the scaling behavior with this universal (parameter-free) form, together with the observed square-root growth of the clone width over the same period (Fig. 2D, inset), reveals that stem cells are indeed being replaced laterally by neighboring stem cells in an effectively one-dimensional geometry. This behavior can accommodate both variability in replacement rates and potential stem cell replacement parallel to the crypt axis (SOM S-III.4 and S-III.5).

Because this pattern of stem cell fate emerges from the consideration of (universal) short-time dynamics, the long-term behavior, including the

drift toward clonality, presents a powerful test of the model. By fixing just a single parameter, $\lambda/N_{\text{stem}}^2 = 0.025 \pm 0.003$ per week (mean $\pm$ SEM), from a quantitative fit to the average clone width (Fig. 1M), we obtained an excellent agreement of theory (detailed in SOM S-IV and S-VI) with the measured monoclonal fraction (Fig. 1K) and clone size distributions over the entire time course (Fig. 3, C to H, and fig. S2). A similar analysis of clone fate as measured from the crypt base in the colon reveals the same neutral drift behavior (Fig. 3, I and J, and SOM S-VI).

These results reveal that stem cells of the small intestine and colon behave as an equipotent population following a pattern of neutral drift in which the loss of a stem cell is compensated by the multiplication of a neighbor. This process may be achieved through stochastic stem cell loss triggering self-renewal, or through overcrowding of the stem cell pool leading to loss. Further, anal-

ysis of sister cell orientation reveals that the frequent transverse cell divisions required for stem cell replacement occur at the crypt base (fig. S3).

Which cells constitute the stem cells, and what is their rate of loss? Current debate centers on the relationship between two crypt base populations, the Bmi1^+ cells positioned around row 4 (4) and the columnar Lgr5^+ cells that reside at the crypt base (5, 19). Because both cell populations support long-lived clonal progeny, equipotency requires that Bmi1^+ and Lgr5^+ cells contribute to the same stem cell pool, with the cells generating each other. Such heterogeneity would not affect the scaling behavior in Eq. 2, as its effect would be resolved rapidly compared to one-dimensional drift around the crypt circumference (SOM S-III.4). If we estimate the number of stem cells on the basis of the total number of Bmi1^+ and Lgr5^+ cells (4, 5, 19), we can conclude that their total number is >16 , suggesting that stem cells are replaced laterally by

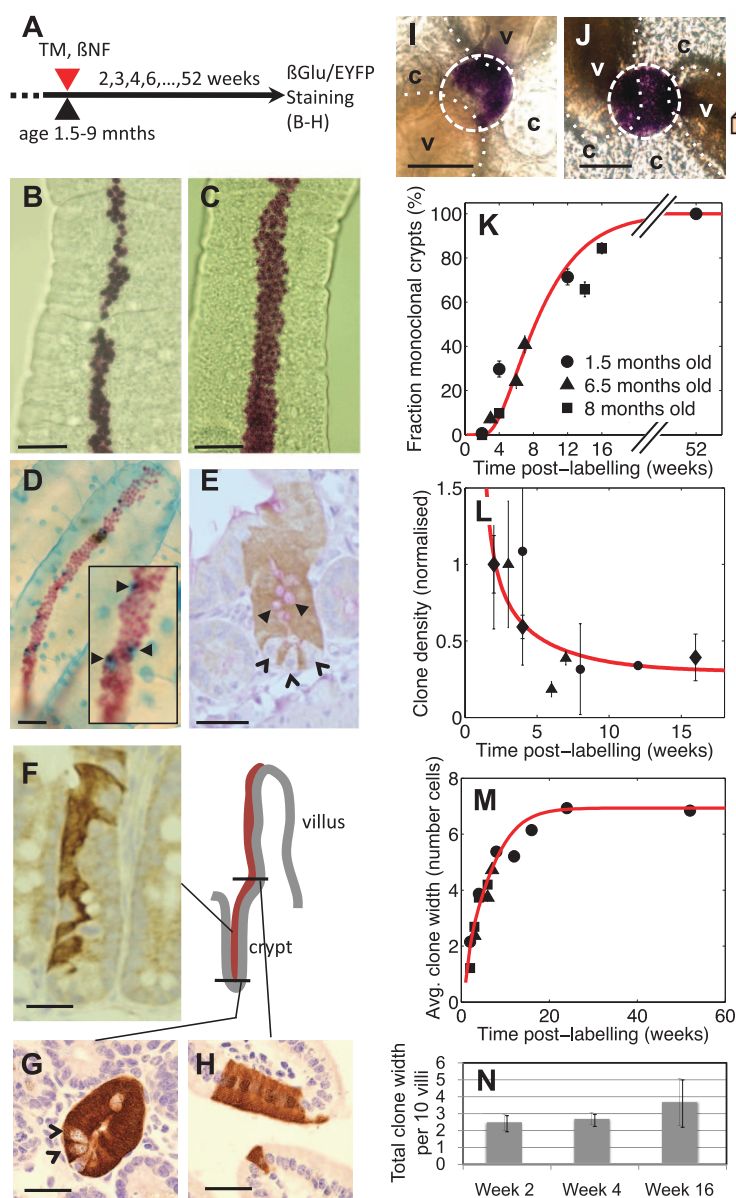


Fig. 1. Conversion to monoclonality implies that stem cells are replaced in mouse intestinal crypts. (A) Experimental schedule; clones were induced by a single pulse of β -naphthoflavone (BNF) and tamoxifen (TM) in adult mice aged 1.5 to 9 months, and then visualized in the small intestine and colon after chase periods of 2 weeks to 1 year through serial sectioning and whole-mount imaging. (B and C)

Clonal progeny migrate in coherent streams on villi in whole-mounted tissue, emerging from partially labeled (B) and fully labeled crypts (C). Streams from single crypts are seen to split to occupy one (shown) or up to three villi (not shown). (D and E) Clones contain both enterocytes and Goblet cells. (D) Whole mount containing βglu^+ clone stained with Alcian blue to visualize Goblet cells (arrowheads); (E) a sectioned EYFP+ (enhanced yellow fluorescent protein) clone stained with Periodic acid-Schiff to visualize Goblet cells (full arrowheads). Positive Paneth cells are initially absent (v-arrows) but ultimately become labeled (G). (F to H) Schematic shows crypt-to-villus axis of clonal migration streams (brown) on the intestinal epithelium (gray). (F) Longitudinal section of a 2-week-old clone; (G and H) serial sections of a clone migration stream showing a labeled 8-week-old clone. Paneth cells are labeled (v-arrows). (I and J) Whole-mounted tissue showing a partly labeled and a fully labeled crypt, respectively. Dashed circles indicate crypt boundaries; dotted lines show villi (v) out of the focal plane; "c" marks adjacent crypts. Diagram shows the basal viewing perspective of the crypts (circles), of which one is clonally labeled (purple). (K) The fraction of fully labeled crypts over time shows conversion to monoclonality. The line shows a fit to neutral drift dynamics (see main text). Monoclonal conversion occurs at comparable rates, irrespective of age at induction (legend). (L) The number of labeled crypts per 10^4 villi decays over time after labeling. Densities are normalized by their earliest time point (2 and 3 weeks, respectively) to allow comparison of different cohorts [legend as in (K)]. (M) The average clone width increases during monoclonal conversion at all ages [legend as in (K)]. Theoretical curves in (J) and (K) follow from the fit made in (K). (N) Representative stem cell labeling; the total labeled cross section of villi remains constant, indicating that the labeled cells maintain a constant population (error bars, SEM). Scale bars, 25 μm .

Fig. 2. Short-time clone size distributions reveal a pattern of “neutral drift” in stem cell replacement. **(A and B)** Models of stem cell turnover in crypt: In **(A)**, monoclonal conversion follows from turnover of a single, slow-cycling, asymmetrically dividing “master” stem cell at the crypt base. Blue arrows show self-renewal through asymmetric division; gray arrows show divisions leading to differentiation and upward migration. In **(B)**, conversion arises from turnover of an equipotent stem cell population in which stem cell loss is compensated by the multiplication of other stem cells (blue arrows), resulting in random clonal expansion and contraction. **(C)** Distribution of clone widths between 2 and 52 weeks after labeling (each “+” translates to one clone). **(D)** When plotted against $w/\langle w(t) \rangle$, the short-term ($t \leq 4$ weeks) clone size distributions, $\langle w(t) \rangle P_w(t)$, show the collapse predicted by the scaling Eq. 1. A log-log plot of the average clone width $\langle w(t) \rangle$ (inset) shows square-root growth, $\log(\langle w(t) \rangle) = 0.5 \log(t - T_f)$ irrespective of age (legend). $T_f \approx 1$ week is the migration time from the crypt base onto the villi (23). The coincidence of the data with the scaling function Eq. 2 (curve), together with the square-root growth of $\langle w(t) \rangle$, provides a signature of one-dimensional neutral drift dynamics (see main text).

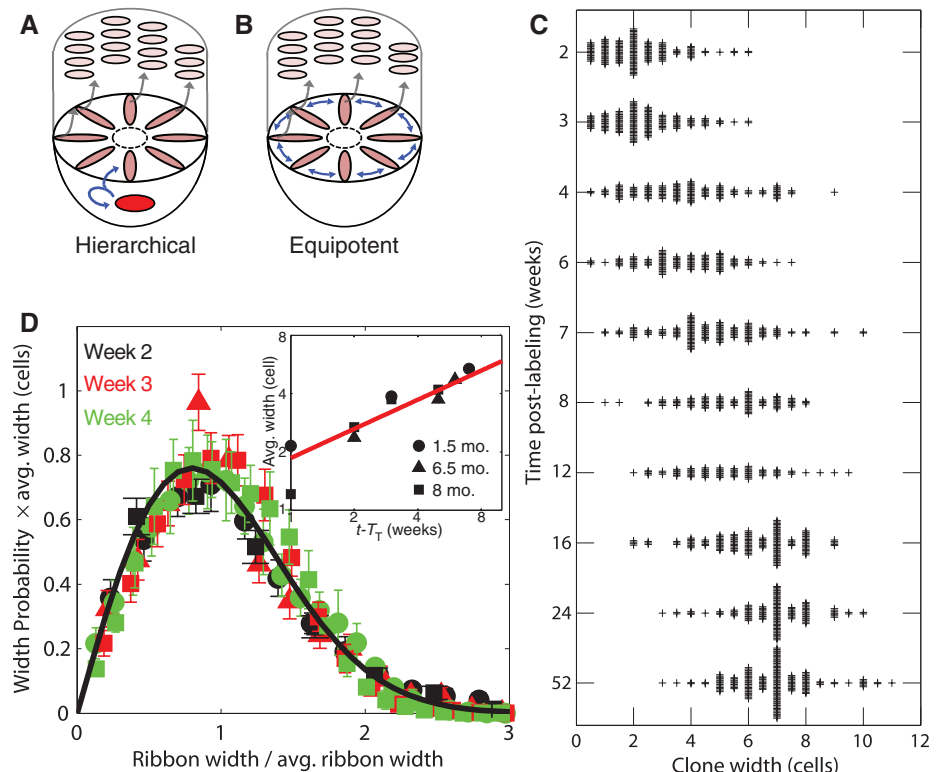
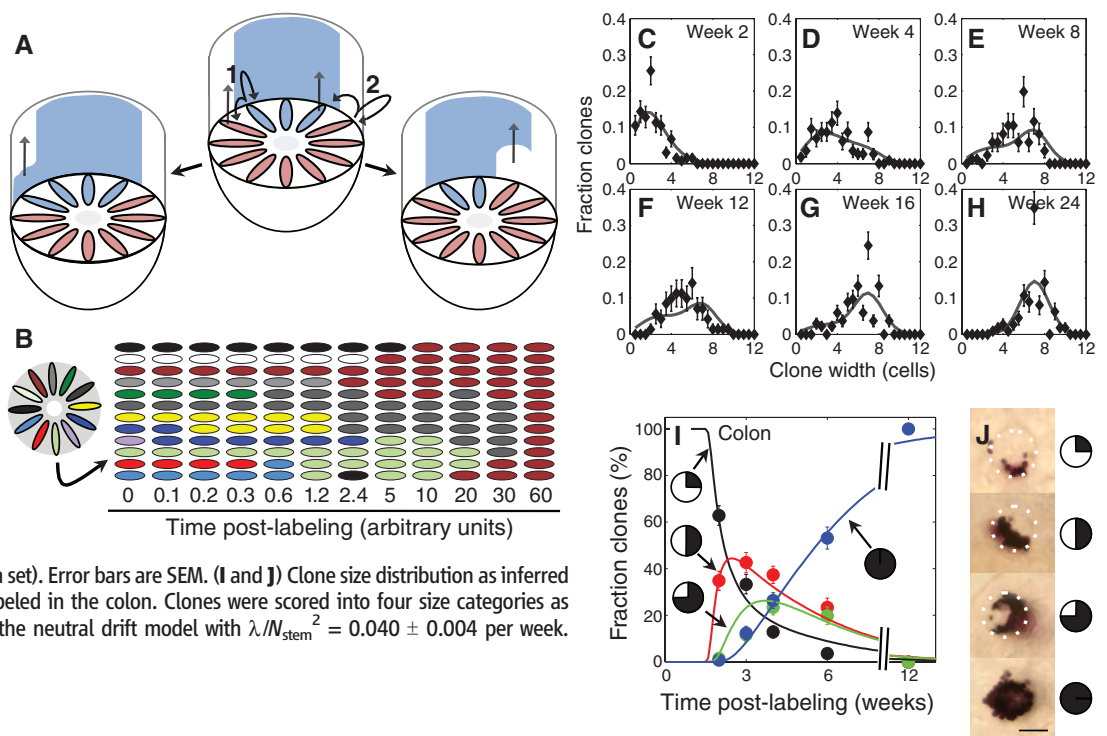


Fig. 3. Model of neutral drift and monoclonal conversion in the small intestine and colon. **(A)** The neutral drift model in which N_{stem} equivalent stem cells surround the crypt base. The chance loss of a stem cell and its replacement at the clone edge leads either to the expansion of the labeled (blue) clone (case 1) or to its contraction (case 2). **(B)** A simulation based on this neutral drift model showing monoclonal conversion after the labeling of all stem cells by different colors. **(C to H)** Clonal width distributions up to 6 months after labeling alongside theoretical curves following from the neutral drift model with $\lambda/N_{\text{stem}}^2 = 0.025$ per week (see fig. S2 for entire data set). Error bars are SEM. **(I and J)** Clone size distribution as inferred from the fraction of crypt base labeled in the colon. Clones were scored into four size categories as shown in **(J)**. Curves follow from the neutral drift model with $\lambda/N_{\text{stem}}^2 = 0.040 \pm 0.004$ per week. Scale bars, 25 μm .



their neighbors at a rate $\lambda \approx 1$ per day comparable to the measured cell division rate (5, 20–22). Therefore, asymmetric cell division is not the sole, or even the most common, mode of stem cell division in the intestine: Symmetric stem cell division is not a rare event, but is a central aspect of homeostasis.

In place of a hierarchical arrangement, our results identify a pool of equipotent stem cells that is regulated by the behavior of neighbors. The pattern of stem cell regulation in the intestine provides an inherently flexible assembly in which any stem cell can be deployed to differentiate into one of a number of cell types, act to replace stem

cells locally, and respond to changing environmental demand.

References and Notes

1. F. M. Watt, B. L. Hogan, *Science* **287**, 1427 (2000).
2. S. J. Morrison, J. Kimble, *Nature* **441**, 1068 (2006).
3. D. H. Scoville, T. Sato, X. C. He, L. Li, *Gastroenterology* **134**, 849 (2008).

4. E. Sangiorgi, M. R. Capecchi, *Nat. Genet.* **40**, 915 (2008).
5. N. Barker *et al.*, *Nature* **449**, 1003 (2007).
6. C. S. Potten, G. Owen, D. Booth, *J. Cell Sci.* **115**, 2381 (2002).
7. A. J. Quyn *et al.*, *Cell Stem Cell* **6**, 175 (2010).
8. D. J. Winton, M. A. Blount, B. A. Ponder, *Nature* **333**, 463 (1988).
9. D. F. Griffiths, S. J. Davies, D. Williams, G. T. Williams, E. D. Williams, *Nature* **333**, 461 (1988).
10. H. S. Park, R. A. Goodlad, N. A. Wright, *Am. J. Pathol.* **147**, 1416 (1995).
11. C. Booth, C. S. Potten, *J. Clin. Invest.* **105**, 1493 (2000).
12. M. Loeffler, A. Birke, D. Winton, C. Potten, *J. Theor. Biol.* **160**, 471 (1993).
13. M. Loeffler, T. Bratke, U. Paulus, Y. Q. Li, C. S. Potten, *J. Theor. Biol.* **186**, 41 (1997).
14. D. J. Winton, B. A. Ponder, *Proc. Biol. Sci.* **241**, 13 (1990).
15. M. Bjerknes, *Biophys. J.* **48**, 85 (1985).
16. E. D. Williams, A. P. Lowes, D. Williams, G. T. Williams, *Am. J. Pathol.* **141**, 773 (1992).
17. A. M. Klein, T. Nakagawa, *Cell Stem Cell* **7**, 214 (2010).
18. A. M. Klein, D. P. Doupe, P. H. Jones, B. D. Simons, *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **76**, 021910 (2007).
19. N. Barker *et al.*, *Cold Spring Harb. Symp. Quant. Biol.* **73**, 351 (2008).
20. H. S. Al-Dewachi, N. A. Wright, D. R. Appleton, A. J. Watson, *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* **18**, 225 (1975).
21. H. S. Al-Dewachi, D. R. Appleton, A. J. Watson, N. A. Wright, *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* **31**, 37 (1979).
22. C. S. Potten, *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **49**, 257 (1986).
23. N. A. Wright, M. Irwin, *Cell Tissue Kinet.* **15**, 595 (1982).
24. We thank H. Zecchini and R. Kemp at Cancer Research UK, Cambridge Research Institute (CRI), and the CRI Biological Resource Unit and Histopathology Core. This

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Materials and Methods

SOM Text

Figs. S1 to S3

References

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Mutational Robustness of Ribosomal Protein Genes

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The distribution of fitness effects (DFE) of mutations is of fundamental importance for understanding evolutionary dynamics and complex diseases and for conserving threatened species. DFEs estimated from DNA sequences have rarely been subject to direct experimental tests. We used a bacterial system in which the fitness effects of a large number of defined single mutations in two ribosomal proteins were measured with high sensitivity. The obtained DFE appears to be unimodal, where most mutations (120 out of 126) are weakly deleterious and the remaining ones are potentially neutral. The DFEs for synonymous and nonsynonymous substitutions are similar, suggesting that in some genes, strong fitness constraints are present at the level of the messenger RNA.

The distribution of fitness effects (DFE) of mutations is important in evolutionary biology; for example, in the degradation of genetic information due to Muller's ratchet (1), molecular clocks (2), genetic variation at the molecular level (3), and the impact of selection and genetic drift in natural populations (4). The DFE is also of importance for understanding quantitative traits, complex diseases, the evolution of antibiotic resistance, and predicting the minimal populations sizes needed for maintaining healthy populations of endangered species and in breeding programs (5–8). Mutations may be deleterious, neutral, or beneficial, and the proportion of mutations in each category will depend on several factors (9). Advantageous mutations are rare and appear to be exponentially distributed (6, 10–12). The DFE of deleterious mutations often appears to be bimodal, with one low-fitness peak (including lethal mutations) and a second peak close to wild-type fitness, with weakly deleterious mutations (13). Information on the DFE for deleterious mutations comes mostly from analyses of DNA sequence data (2, 5, 14) or mutation accu-

mulation and mutagenesis experiments (15–18). The indirect estimates of fitness values made from sequence data suffer the drawback that strongly deleterious mutations (with $|s|N_e \gg 1$) ($|s|$, absolute value of selection coefficient; N_e , effective population size) are poorly represented, and many details of the DFE are unresolved (19). Direct measurements, on the other hand, are difficult at the high-fitness end, where deleterious effects are

smaller than the percent level (in this study, selection coefficients $|s| < 3 \times 10^{-3}$ could not be detected). Nevertheless, experimental studies of the fitness effects of defined single-point mutations have proven useful, because they allow an assumption-free determination of the underlying DFE, including the frequencies of strongly deleterious mutations. A few such studies in viruses have demonstrated a bimodal DFE, with most mutations being either neutral or lethal (13, 20, 21).

We used *Salmonella typhimurium* to study the DFE of random base-pair substitutions in the protein synthesis machinery. A total of 126 random base-pair substitutions were engineered into the *rpsT* and *rplA* genes, encoding the ribosomal proteins S20 and L1, respectively (22). These two proteins are nonessential, but deletion mutants lacking either of these ribosomal proteins have severely reduced fitness. Thus, putative mutational effects on fitness can be measured over a large range, and the fitness of complete loss-of-function mutations is known and is larger than zero. We used bacterial growth rate to measure the fitness effects of the mutations. The involvement of ribosomal proteins in translation and the direct relation of translation rates to exponential growth

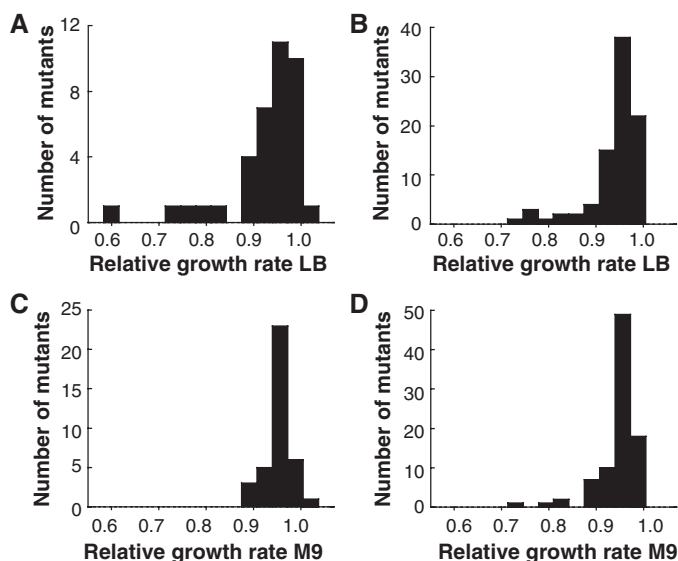


Fig. 1. Relative exponential growth rates of single-substitution mutants relative to wild-type controls set to 1. (A) Synonymous substitutions (in LB). (B) Nonsynonymous substitutions (in LB). (C) Synonymous substitutions (in M9 medium). (D) Nonsynonymous substitutions (in M9 medium).

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rates (23) ensure that fitness effects will be directly correlated to the quality and quantity of available ribosomes.

In total, 70 single base-pair mutants of *rpsT* (S20), 56 mutants of *rplA* (L1), and four independent wild-type controls for each gene were constructed. The 126 random mutations comprised 38 synonymous base-pair substitutions and 88 nonsynonymous substitutions. The identity and fitness data of the individual mutants are available as supporting online material (SOM) (tables S1 to S3). Exponential growth rates were reduced for most of the substitution mutants, with an average relative growth rate of 0.92 (range, 0.61 to 1.01) for synonymous and 0.94 (0.72 to 1.00) for nonsynonymous mutants in rich growth medium (LB), as compared to wild-type controls (relative growth rate set to 1) and a median of 0.96 and 0.95, respectively (Fig. 1, A and B, and figs. S1 and S2). In the poorer M9 glucose minimal medium, the average growth rates were 0.95 (median 0.96) for both synonymous (0.89 to 1.01) and nonsynonymous (0.72 to 1.00) substitutions (Fig. 1, C and D, and figs. S3 and S4). As expected, no significantly advantageous mutations were found. No mutations caused a complete loss of function, and the mutants with the most pronounced fitness reduction (0.61 and 0.74) were still much more fit than the deletion mutants, which showed a relative fitness of 0.29 (*rpsT*, S20) and 0.25 (*rplA*, L1) in LB (24).

The growth rate measurements have limited sensitivity, detecting changes in $s > 0.03$ (3%), and measure fitness only in the exponential growth phase. To increase sensitivity, we performed competition experiments (24), in which we measured a composite fitness during the entire growth curve. In these competitions, sensitivity is much improved, allowing the detection of fitness differences as small as $s = 0.003$ (0.3%) (24). The average selection coefficients in this experimental setup were -0.0096 (-0.0279 to 0 , Fig. 2A) for synonymous and -0.0131 (-0.0763 to 0 , Fig. 2B) for nonsynonymous substitutions. The large majority of the mutations, including synonymous substitutions, caused significant fitness costs that confer strong counterselection (25). Mutations were evenly distributed throughout the genes, and no correlation between gene position and fitness was found (Fig. 3, A and B). The DFEs (for $-s$) of the mutations were estimated by fitting of commonly used univariate distributions (13) to the data using a maximum-likelihood method (Fig. 2, C and D, figs. S7 to S14, table S5, and SOM text).

To explain the similarities in DFEs for synonymous and nonsynonymous changes, we assume that the mechanistic cause(s) of the fitness effects are the same, especially if the effects of most amino acid changes are generally very small but occasionally large, accounting for the outliers in the distribution of nonsynonymous substitutions. This idea is supported by the absence of any significant correlation between fitness and predicted changes in protein free energy (Fig. 3C),

conservation (Fig. 3D), and ribosomal RNA contacts (table S4) for the whole set of nonsynonymous mutations. Instead, our data suggest that the fitness reduction produced by base-pair substitutions is primarily conferred by changes in mRNA,

which could be manifested at several levels, including, for example, suboptimal codon usage or altered mRNA structure and/or stability. The former is unlikely for several reasons. First, even though several of the synonymous mutations with large

Fig. 2. Selection coefficients of single-substitution mutants relative to isogenic wild-type controls (defined as $s = 0$, SD = 0.001 within experiment variation for competition controls) during growth in M9 medium. (A) Synonymous substitutions. (B) Nonsynonymous substitutions. (C) Cumulative distribution function of fitness costs ($-s$) for synonymous substitutions, with x's showing the experimental data and the line showing a fitted gamma function. (D) Cumulative distribution function of fitness costs ($-s$) for nonsynonymous substitutions, with x's showing the experimental data and the line showing a fitted gamma function.

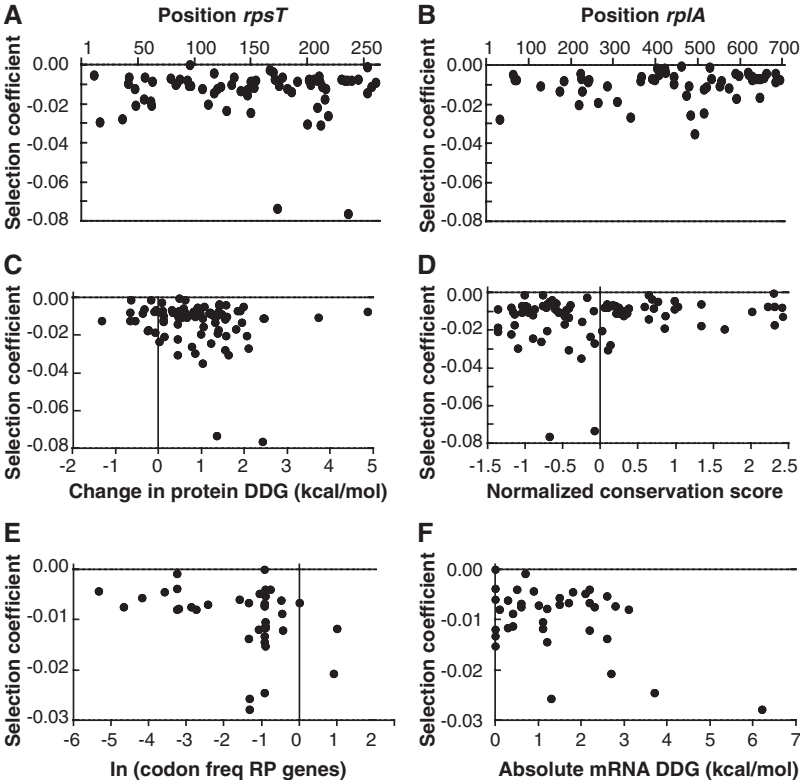
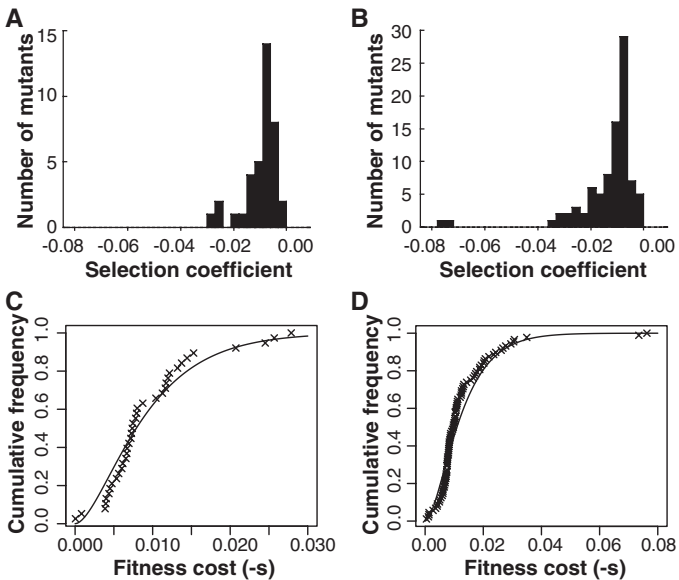


Fig. 3. Analyses of fitness costs. (A) Position of substitutions in *rpsT* (S20) and fitness costs. (B) Position of substitutions in *rplA* (L1) and fitness costs. (C) Influence of changes in predicted protein free energy (DDG is destabilizing) on fitness costs of nonsynonymous substitutions. (D) Evolutionary conservation versus fitness costs for nonsynonymous mutations. (E) Logarithmic ($\ln$) change in the frequency of codons used in ribosomal protein (RP) genes for synonymous substitutions. (F) Absolute changes in predicted mRNA free energy versus fitness costs for synonymous substitutions.

fitness costs involve more rarely used codons, this is true for only a limited subset of mutations (Fig. 3E and table S4). Second, we could not find any overall correlation between relative codon usage and fitness effects, using either all *S. typhimurium* codons or only those used in ribosomal protein genes (Fig. 3E and table S4). This is also consistent with our previous experimental work in which substitution of the *S. typhimurium rpsT* and *rplA* genes with genes from other species with different codon usage caused very small fitness effects (24).

These small fitness costs suggest that the fitness constraints on the mRNA for the two ribosomal protein genes are highly conserved between related bacterial species and that this functional conservation is largely independent of codon usage. Selection coefficients determined from the competition experiments were plotted as a function of the absolute values of the predicted free energy change for the mRNA of the synonymous mutants. A weak but significant correlation [correlation coefficient (r) = 0.47, P = 0.0027, n = 38 synonymous mutants] was found, indicating a general connection between changed mRNA structure and fitness (Fig. 3F and table S5). However, no significant changes in mRNA levels could be detected by quantitative real-time fluorescence polymerase chain reaction for synonymous mutants with large fitness costs (SOM text). Studies of synonymous substitutions usually involve large changes in codon usage or particular examples of substitutions with large effects. Mutagenesis studies of single proteins rarely include the use of high-sensitivity assays of fitness and analysis of synonymous substitutions (SOM references).

Studies of fitness effects of defined base substitutions in viruses have focused on the DFE at the whole-genome level, whereas we studied two specific bacterial genes. However, the viruses examined are small and encode only 5 to 11 genes, meaning that there are many independently engineered mutations for each virus gene and that the DFE can also be studied at the level of the individual genes (13, 20, 21). Comparing the shape of the distributions obtained here with those from similar experiments in viruses reveals two differences that are valid both when the viral DFEs are analyzed at the level of the whole genome and of individual genes. First, for viruses, the most frequently found mutational type was lethal (up to 40%) (13, 20, 21), whereas most of the mutations examined here had only small effects on fitness (91% had s values between -0.003 and -0.03). Thus, the compact virus genomes appear to be highly constrained with regard to which sequence changes are acceptable for phage viability (13).

The second difference is the rarity of apparently neutral mutations found here as compared to the viruses examined (13, 20, 21). For the ribosomal protein genes, 6 of 126 mutants (4.8%) had $|s| < 0.003$, whereas for the viruses, 25% appeared neutral. One reason for this difference could be that the higher sensitivity of our fitness

assays allows mutations with small fitness effects to be distinguished from neutral mutations and that a similar peak of weakly deleterious mutations might also exist in the viral systems. Thus, it is conceivable that the relatively high frequencies of apparently neutral mutations observed in certain experimental systems (13, 20, 21) are mainly a consequence of the limited sensitivity of the assays and that the proportion of deleterious mutations is very high even when synonymous substitutions are included.

References and Notes

1. D. Butcher, *Genetics* **141**, 431 (1995).
2. G. Piganeau, A. Eyre-Walker, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 10335 (2003).
3. B. Charlesworth, M. T. Morgan, D. Charlesworth, *Genetics* **134**, 1289 (1993).
4. T. Ohta, *Annu. Rev. Ecol. Syst.* **23**, 263 (1992).
5. A. Eyre-Walker, M. Woolfit, T. Phelps, *Genetics* **173**, 891 (2006).
6. R. Kassen, T. Bataillon, *Nat. Genet.* **38**, 484 (2006).
7. A. Caballero, P. D. Keightley, *Genetics* **138**, 883 (1994).
8. L. Perfeito, L. Fernandes, C. Mota, I. Gordo, *Science* **317**, 813 (2007).
9. A. Eyre-Walker, P. D. Keightley, *Nat. Rev. Genet.* **8**, 610 (2007).
10. D. R. Rokytka, P. Joyce, S. B. Caudle, H. A. Wichman, *Nat. Genet.* **37**, 441 (2005).
11. J. H. Gillespie, *Evolution* **38**, 1116 (1984).
12. H. A. Orr, *Genetics* **163**, 1519 (2003).

13. R. Sanjuán, A. Moya, S. F. Elena, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 8396 (2004).
14. R. Nielsen, Z. Yang, *Mol. Biol. Evol.* **20**, 1231 (2003).
15. S. Trindade, L. Perfeito, I. Gordo, *Philos. Trans. R. Soc. London B Biol. Sci.* **365**, 1177 (2010).
16. T. Mukai, *Genetics* **50**, 1 (1964).
17. D. M. Wloch, K. Szafraniec, R. H. Borts, R. Korona, *Genetics* **159**, 441 (2001).
18. E. K. Davies, A. D. Peters, P. D. Keightley, *Science* **285**, 1748 (1999).
19. P. D. Keightley, A. Eyre-Walker, *Philos. Trans. R. Soc. London B Biol. Sci.* **365**, 1187 (2010).
20. P. Carrasco, F. de la Iglesia, S. F. Elena, *J. Virol.* **81**, 12979 (2007).
21. P. Domingo-Calap, J. M. Cuevas, R. Sanjuán, D. J. Begun, *PLoS Genet.* **5**, e1000742 (2009).
22. Materials and methods are available as supporting material on Science Online.
23. M. Ehrenberg, C. G. Kurland, *Q. Rev. Biophys.* **17**, 45 (1984).
24. P. A. Lind, C. Tobin, O. G. Berg, C. G. Kurland, D. I. Andersson, *Mol. Microbiol.* **75**, 1078 (2010).
25. M. Kimura, *Genetics* **47**, 713 (1962).
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Supporting Online Material

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Tables S1 to S7
References

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Suppression of Antitumor Immunity by Stromal Cells Expressing Fibroblast Activation Protein- α

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The stromal microenvironment of tumors, which is a mixture of hematopoietic and mesenchymal cells, suppresses immune control of tumor growth. A stromal cell type that was first identified in human cancers expresses fibroblast activation protein- α (FAP). We created a transgenic mouse in which FAP-expressing cells can be ablated. Depletion of FAP-expressing cells, which made up only 2% of all tumor cells in established Lewis lung carcinomas, caused rapid hypoxic necrosis of both cancer and stromal cells in immunogenic tumors by a process involving interferon- γ and tumor necrosis factor- α . Depleting FAP-expressing cells in a subcutaneous model of pancreatic ductal adenocarcinoma also permitted immunological control of growth. Therefore, FAP-expressing cells are a nonredundant, immune-suppressive component of the tumor microenvironment.

Almost 20 years ago, an important advance in tumor immunology was the discovery that a human melanoma may express an unmutated tumor-associated antigen that spontaneously elicits a CD8⁺ T cell response (1). However, therapeutic vaccination with such antigens has only rarely been effective in controlling tumor growth. Some studies suggest that cancers induce systemic tolerance (2) or lose antigen expression as they progress (3, 4), but these explanations cannot account for the findings that systemic immune responses occur in patients immunized with such antigens (5, 6) and that these

responses do not induce or maintain tumor regression, despite persistent expression of antigen and major histocompatibility complex (MHC) class I

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by tumors (5). These observations indicate that immune suppression within the tumor microenvironment may be a major determinant of the poor outcome of therapeutic vaccination.

Although suppression may be mediated by cancer cells (7), “the paradoxical finding that antigenically foreign cell clones can develop into a tumor in an animal and are not automatically eliminated by the immune response” (8), as well as the occurrence of concomitant immunity (9), indicates that stromal cells have a major role in immune suppression. Of the two general types of nonvascular stromal cells, hematopoietic and mesenchymal, the former, which includes myeloid-derived suppressor cells (10, 11), M2 macrophages (12), certain natural killer T cells (13), and CD4⁺ Foxp3⁺ regulatory T cells (14), has been considered more often in this context than mesenchymal cells, which have usually been studied as human carcinoma-associated fibroblasts in xenografted, immune-deficient mice (15). Nevertheless, a tumoral stromal cell of apparent mesenchymal origin—identifiable by its expression of the type II membrane dipeptidylpeptidase fibroblast activation protein- α (FAP) (16)—is associated with other biological processes in which immune suppression may occur, such as the gravid uterus and chronic, noninfected inflammatory lesions (17, 18).

To assess the immune suppressive function of FAP⁺ stromal cells in a tumoral microenvironment, we created two transgenic (Tg) mouse lines with bacterial artificial chromosomes (BACs) containing the murine *fap* gene modified by insertion of a cassette encoding either enhanced green fluorescent protein (EGFP) or the primate diphtheria toxin receptor (DTR) (fig. S1) (19). We subcutaneously injected EGFP Tg mice with cells of the LL2 Lewis lung carcinoma line, removed tumors after 16 days, and assessed frozen sections by confocal microscopy. In tumoral stromal cells, there was colocalization of EGFP and staining by an antibody against FAP (anti-FAP), demonstrating that the modified BACs contained the *fap* transcriptional elements necessary for appropriate expression of the inserted cassettes (Fig. 1A). Further characterization of EGFP⁺ stromal cells by confocal microscopy showed that they are composed of both CD45⁺ and CD45[−] cells, all staining with antibody to α -smooth muscle actin (α -SMA) and some with antibody to collagen type I (Col I) (Fig. 1A). Phenotyping FAP⁺ stromal cells by flow cytometry revealed that the CD45[−] subset expressed CD34 and Sca-1 and that the CD45⁺ subset expressed CD11b, MHC class II, and Sca-1, but not Gr-1 (fig. S2). Thus, the CD45[−] subset shares markers with the mesenchymal stem cells (MSCs) (20), as do human FAP⁺ cells (21), and the CD45⁺ subset resembles the CD11b⁺/class II⁺/Col I⁺/ α -SMA⁺ fibrocyte (22). In LL2 tumors rendered immunogenic by expression of ovalbumin (LL2/OVA), CD45[−] and CD45⁺ FAP⁺ stromal cells each made up ~1% of all tumoral cells; the LL2/OVA cells and CD45⁺/FAP[−] cells represented 66 and 30% of the cells, respectively (Fig. 1B). We also found

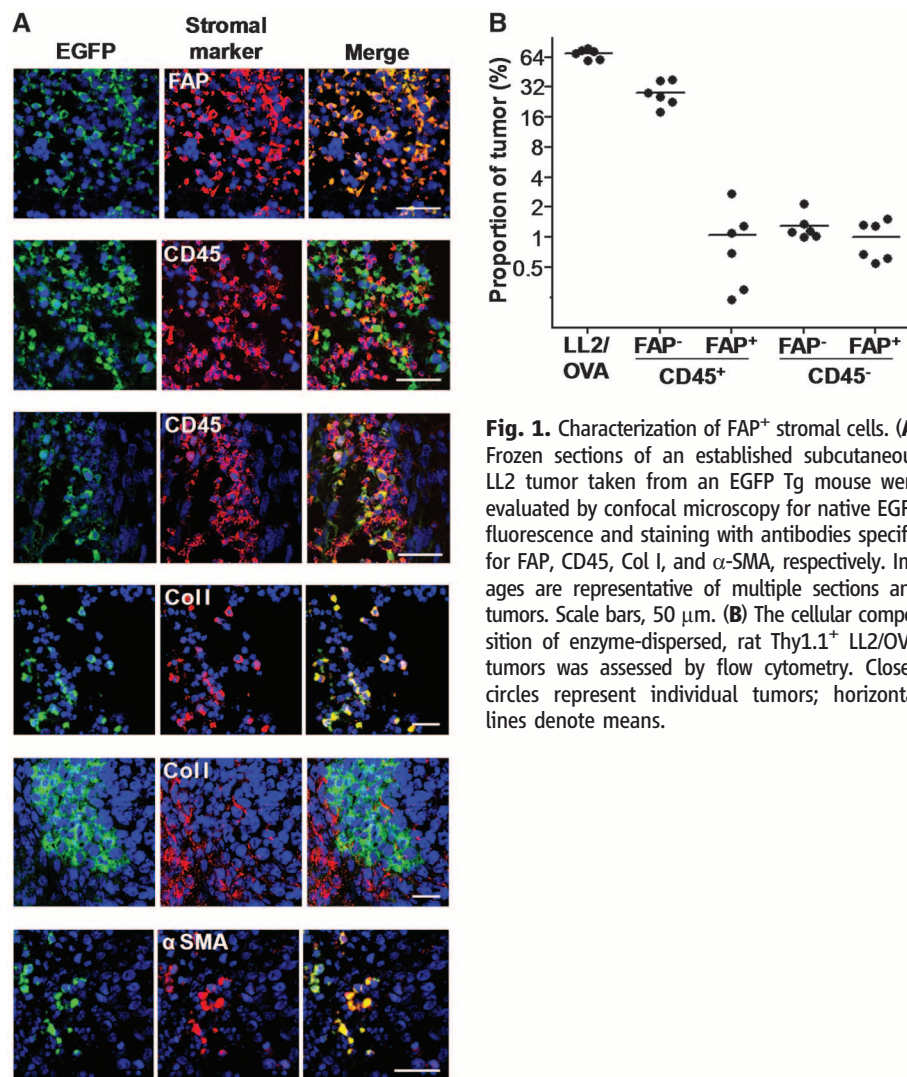


Fig. 1. Characterization of FAP⁺ stromal cells. (A) Frozen sections of an established subcutaneous LL2 tumor taken from an EGFP Tg mouse were evaluated by confocal microscopy for native EGFP fluorescence and staining with antibodies specific for FAP, CD45, Col I, and α -SMA, respectively. Images are representative of multiple sections and tumors. Scale bars, 50 μ m. (B) The cellular composition of enzyme-dispersed, rat Thy1.1⁺ LL2/OVA tumors was assessed by flow cytometry. Closed circles represent individual tumors; horizontal lines denote means.

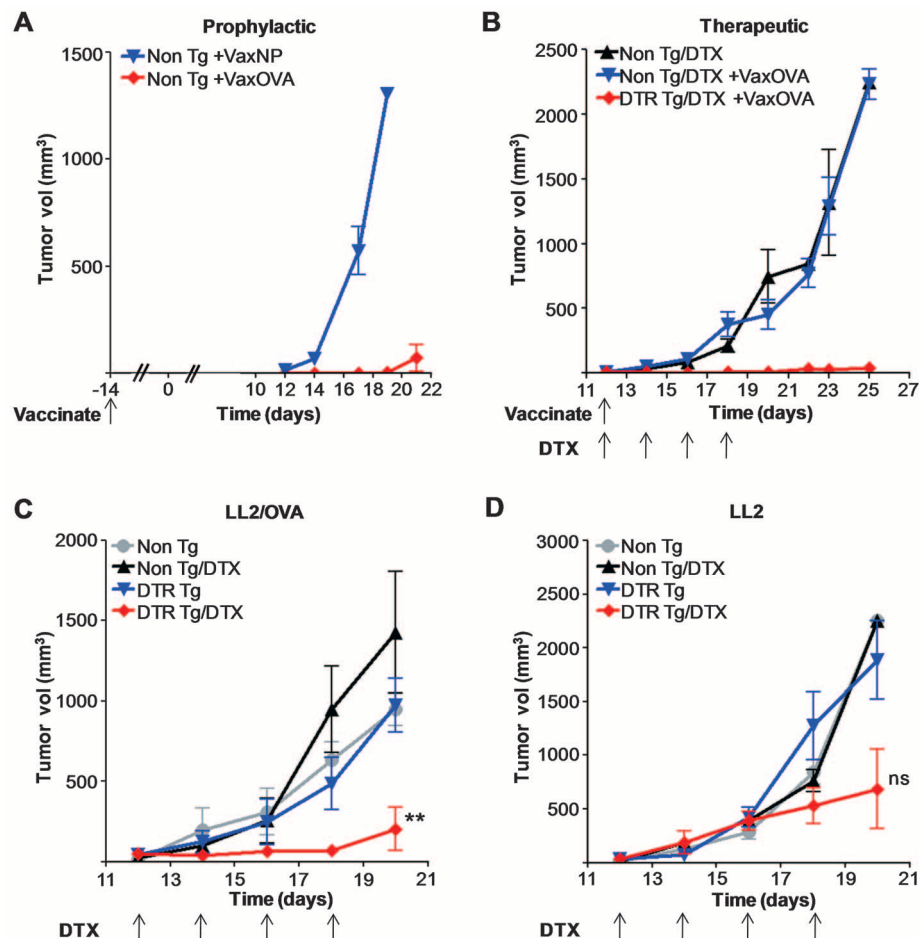
CD45⁺ and CD45[−] FAP⁺ cells in the bone marrow (fig. S3A), which is the origin of MSCs and fibrocytes. The functionality of the DTR Tg was shown by the decreased number of FAP⁺ cells in both LL2/OVA tumors and bone marrow from DTR Tg mice after treatment with diphtheria toxin (DTX) (fig. S3). The loss of FAP⁺ stromal cells did not abolish tumoral FAP expression because LL2 cells express FAP (fig. S2A), which is relevant because inhibition of FAP peptidase activity can impair nonimmunogenic tumor growth (23).

To determine if FAP⁺ stromal cells contribute to the resistance of an immunogenic tumor to therapeutic vaccination, we first validated the efficacy of prophylactic vaccination. We immunized non-Tg mice with vaccinia virus-expressing OVA (VaxOVA) or influenza nucleoprotein (VaxNP) 2 weeks before subcutaneous injection of LL2/OVA cells. LL2/OVA outgrowth was delayed only in mice that had received VaxOVA, showing that prophylactic immunization is effective (Fig. 2A). Three groups of mice were assessed for the efficacy of therapeutic immunization on day 12 when tumors were palpable: non-Tg mice that did or did not re-

ceive VaxOVA and DTR Tg mice that received VaxOVA. DTX was given to all mice beginning on day 12. Therapeutic vaccination did not slow the growth of established LL2/OVA tumors unless it was combined with FAP⁺ cell ablation, which fully suppressed tumor growth (Fig. 2B). Seven days after therapeutic vaccination, the frequencies of peripheral blood CD8⁺ T cells that were H-2Kb/SIINFEKL(OVA)-specific in non-Tg mice and DTR Tg mice were nearly equivalent ($1.4 \pm 0.5\%$ and $0.7 \pm 0.3\%$, respectively, $P > 0.05$) (fig. S4, A and B), excluding an effect of FAP⁺ cells on the priming of OVA-specific CD8⁺ T cells.

FAP⁺ cell ablation suppressed LL2/OVA growth by 48 hours (Fig. 2B), before a vaccine-induced immune response could have occurred, indicating that either the tumor itself induced an anti-OVA response (the effects of which were locally suppressed by FAP⁺ cells) or the tumor-promoting effect of FAP⁺ stromal cells did not have an immunological basis. To explore these possibilities, we examined the effect of FAP⁺ cell ablation on the growth of established LL2/OVA tumors without therapeutic vaccination. The rate

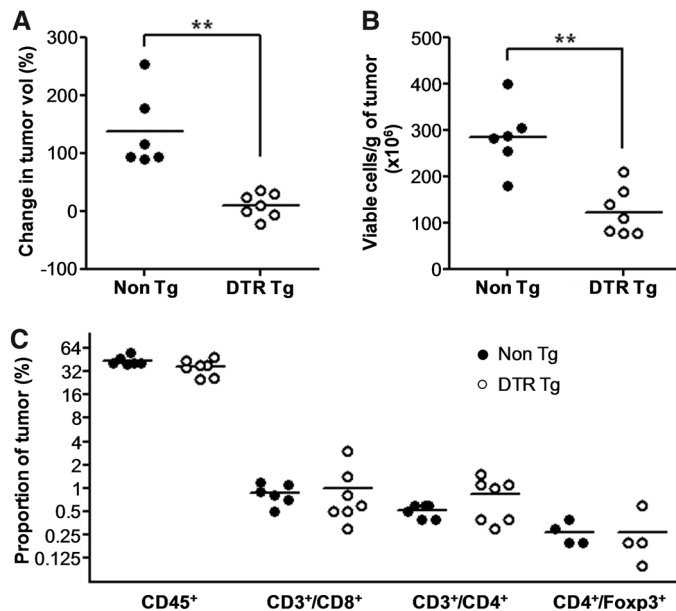
Fig. 2. Combining ablation of FAP⁺ stromal cells with a therapeutic vaccine controls tumor growth. (A) Mice were prophylactically vaccinated with VaxNP or VaxOVA 14 days before subcutaneous injection of LL2/OVA tumors, and tumor sizes were measured thereafter. (B) Non-Tg and DTR Tg mice were injected with LL2/OVA cells. Twelve days later, when tumors were palpable, all mice began alternate-day treatment with DTX, and the indicated groups were therapeutically vaccinated with VaxOVA. (C) Non-Tg and DTR Tg mice were injected with LL2/OVA cells; 12 days later, the indicated groups began alternate-day DTX treatment. (D) Same as in (C), except that mice were injected with LL2 cells. Tumor sizes are presented as mean $\pm$ SEM (error bars). The curves describing tumor growth were compared for differences using the “compareGrowthCurves” permutation test [$**P < 0.01$; not significant (ns), $P > 0.05$; representative of two replicate experiments; cohorts contained four or more mice].



of expansion of LL2/OVA tumors in DTR Tg mice was significantly suppressed by DTX treatment, as compared with that of the control groups of non-Tg mice with or without DTX and DTR Tg mice without DTX (Fig. 2C). Growth arrest was apparent by 48 hours after DTX. At day 20, $0.4 \pm 0.2\%$ and $0.2 \pm 0.2\%$ ($P > 0.05$) of splenic CD8⁺ T cells were H-2Kb/ SIINFEKL(OVA)-specific in the DTX-treated non-Tg mice and DTR Tg mice, respectively (fig. S4C), indicating that the LL2/OVA tumors had induced an immune response, as has been reported by Nelson *et al.* (24). The same analysis of the growth curves of nonimmunogenic LL2 tumors in non-Tg mice and DTR Tg mice with or without DTX did not reveal significant differences (Fig. 2D); however, diminished LL2 growth in DTR Tg mice given DTX did seem to eventually occur after 6 to 8 days of treatment. Therefore, the loss of FAP⁺ stromal cells causes immediate growth arrest of a tumor that has induced an immune response, but not of a nonimmunogenic tumor. Although there may be a nonimmunological function for the FAP⁺ cell, we elected to focus on its immune-suppressive activity because of a potential relation to the poor efficacy of tumor vaccines.

We analyzed LL2/OVA tumors taken from non-Tg and DTR Tg mice 48 hours after initiating DTX to characterize the changes that were

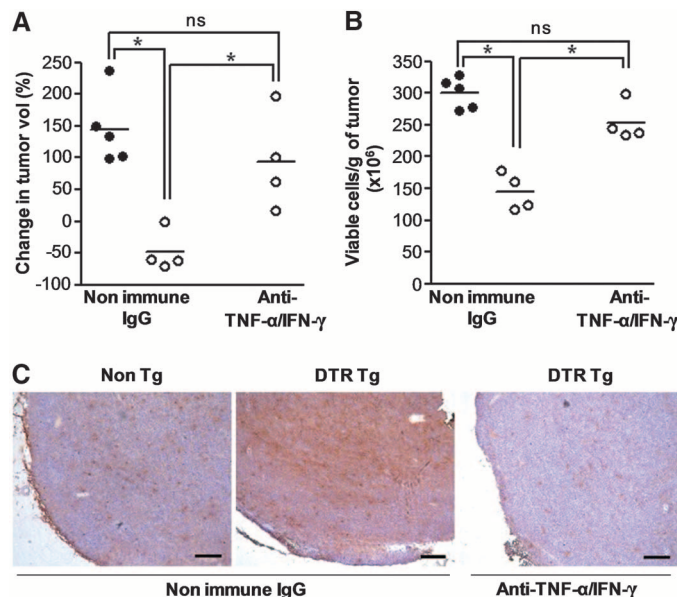
Fig. 3. The acute effects of ablating FAP⁺ stromal cells on LL2/OVA tumors. Non-Tg and DTR Tg mice bearing established LL2/OVA tumors were given DTX; 48 hours later, tumors were assessed for (A) growth by comparison to tumor size before DTX, (B) number of viable cells, and (C) immune cellular composition [$**P < 0.01$; representative of replicate experiments; closed and open circles represent individual tumors; horizontal lines denote means].



specific to the immunogenic tumor. LL2/OVA tumor size in non-Tg mice doubled during this period, whereas growth in the DTR Tg mice ceased (Fig. 3A). Growth arrest was associated with a 60% decrease in the number of viable cells per gram of tumor (Fig. 3B). Loss of viability must have occurred among both LL2/OVA can-

cer cells and CD45⁺ stromal cells, because their relative proportions did not change (Fig. 3C). FAP⁺ cell ablation did not alter the proportions of CD4⁺ or CD8⁺ T cells or of CD4⁺ Foxp3⁺ regulatory T cells, suggesting that tumor cell death did not involve a rapid increase of effector T cells or decrease of suppressive T cells. There was also

Fig. 4. Protection of LL2/OVA tumors from the effects of ablating FAP⁺ stromal cells by neutralizing antibodies to TNF- α and IFN- γ . Non-Tg and DTR Tg mice bearing established LL2/OVA tumors were given control immunoglobulin G (IgG) or neutralizing antibodies to TNF- α and IFN- γ 24 hours before treatment and at 0 hours, the time of the first day of DTX treatment. Forty-eight hours later, tumors were assessed for (A) growth by comparison to size before DTX and (B) number of viable cells (representative of replicate experiments; closed and open circles represent individual tumors from non-Tg and DTR Tg mice, respectively; horizontal lines denote means). (C) Tumors were assessed for the occurrence of hypoxia, as detected in frozen sections by immunoperoxidase staining of stable protein adducts formed with reductively activated pimonidazole (* $P < 0.05$; ns, $P > 0.05$). Scale bar, 200 μ m. Images are representative of multiple sections taken from three mice from each cohort.



no change in the proportion of tumoral CD8⁺ T cells that were both OVA-specific and expressed the activation marker CD69, the cytotoxic molecule granzyme B, or produced interferon- γ (IFN- γ) in response to antigenic stimulation (fig. S5). Taken together, these findings are not consistent with immune suppression of OVA-specific CD8⁺ T cells by FAP⁺ stromal cells. Nevertheless, the absence of arrested growth of LL2/OVA tumors in Rag2-deficient mice depleted of FAP⁺ cells confirms the immunological basis of this response (fig. S6).

Acute hypoxic necrosis secondary to ischemia caused by prothrombotic effects of IFN- γ and tumor necrosis factor- α (TNF- α) is an indirect immunological mechanism that may have been involved in the rapid cell death of LL2/OVA tumors depleted of FAP⁺ cells (25, 26). The presence of mRNA for TNF- α and IFN- γ in the LL2/OVA tumor and the higher amounts of mRNA for IFN- γ and two of its target genes—*IRF-1* (interferon regulatory factor-1) and *iNOS* (inducible nitric oxide synthase)—in LL2/OVA than in LL2 tumors supported this possibility (table S1). Accordingly, non-Tg and DTR Tg mice with established LL2/OVA tumors were given isotype control or neutralizing anti-TNF- α and anti-IFN- γ antibodies during the 48 hours of treatment with DTX, and tumors were then assessed. The impaired tumor growth and decreased recovery of viable tumor cells caused by depleting FAP⁺ cells were largely reversed by anti-TNF- α /anti-IFN- γ treatment (Fig. 4, A and B). The hypoxia occurring in the LL2/OVA tumor after the loss of FAP⁺ cells was also suppressed by anti-TNF- α /anti-IFN- γ treatment (Fig. 4C and fig. S7). Therefore, FAP⁺ stromal cells either suppress the production of TNF- α and IFN- γ , or they attenuate cellular responses

to these cytokines to protect the immunogenic tumor from cytokine-induced hypoxic necrosis. The relatively unchanged expression of these cytokines 48 hours after ablation of FAP⁺ cells would favor the latter explanation (table S1). In addition, there was no marked change in the expression of four potentially immune-suppressive cytokines—transforming growth factor- β 1, interleukin (IL)-4, IL-10, and IL-13—after depletion of FAP⁺ cells (table S1), consistent with the absence of any changes in tumoral CD8⁺ T cell phenotypes.

We determined whether FAP⁺ stromal cells suppress immunological control of another subcutaneous tumor that was established with a cell line derived from a murine pancreatic ductal adenocarcinoma (PDA) arising in the KPC mouse (27). These cancer cells resemble human PDA in many respects, including their expression of oncogenic Kras^{G12D} and the tumor-associated antigen mesothelin, and both spontaneous and subcutaneous tumors contain FAP⁺ stromal cells (fig. S8). When transplanted tumors became palpable, we immunized non-Tg and DTR Tg recipients with a mesothelin peptide. Nine days later, we treated mice with DTX and assessed tumors 48 hours later. Only when depletion of FAP⁺ cells was combined with immunization did the PDA tumor acutely regress (fig. S9A). The immunological basis of this response was demonstrated by its absence in DTR Tg, Rag2-deficient mice that were similarly immunized and depleted of FAP⁺ cells (fig. S9B).

The acute, hypoxic death of both cancer and stromal cells that is observed after FAP⁺ cell ablation is mediated by TNF- α and IFN- γ . These cytokines have previously been shown to be involved in CD8⁺ T cell-dependent killing of

antigen-loss variant tumor cells (28) and the suppression of angiogenesis (29). The finding of a possible relation between FAP⁺ cells and MSCs and fibrocytes, which promote wound healing, is reminiscent of the description of tumors as chronic, nonhealing wounds (30). Therefore, immune suppression by FAP⁺ cells may be a developmentally programmed, tissue-protective function that, in the context of a tumor, is catastrophically inappropriate. Interfering with suppression by FAP⁺ cells of cellular responses to these two cytokines may complement the current most effective form of cancer immunotherapy, the enhancement of lymphocyte activation by antibody to cytotoxic T lymphocyte antigen-4 (31).

References and Notes

1. P. van der Bruggen *et al.*, *Science* **254**, 1643 (1991).
2. G. Willmsky *et al.*, *J. Exp. Med.* **205**, 1687 (2008).
3. G. P. Dunn, C. M. Koebel, R. D. Schreiber, *Nat. Rev. Immunol.* **6**, 836 (2006).
4. D. E. Speiser *et al.*, *J. Immunol.* **177**, 1338 (2006).
5. S. A. Rosenberg *et al.*, *J. Immunol.* **175**, 6169 (2005).
6. D. Valmori *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 8947 (2007).
7. C. Uytendhoe *et al.*, *Nat. Med.* **9**, 1269 (2003).
8. G. Klein, H. O. Sjogren, E. Klein, K. E. Hellstrom, *Cancer Res.* **20**, 1561 (1960).
9. M. J. Berendt, R. J. North, *J. Exp. Med.* **151**, 69 (1980).
10. L. A. Pekarek, B. A. Starr, A. Y. Toledano, H. Schreiber, *J. Exp. Med.* **181**, 435 (1995).
11. I. Marigo, L. Dolcetti, P. Serafini, J. Zanovello, V. Bronte, *Immunol. Rev.* **222**, 162 (2008).
12. A. Mantovani, P. Allavena, A. Sica, F. Balkwill, *Nature* **454**, 436 (2008).
13. M. Terabe *et al.*, *J. Exp. Med.* **202**, 1627 (2005).
14. P. Yu *et al.*, *J. Exp. Med.* **201**, 779 (2005).
15. A. Orimo *et al.*, *Cell* **121**, 335 (2005).
16. P. Garin-Chesa, L. J. Old, W. J. Rettig, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7235 (1990).
17. H. Dolznig *et al.*, *Cancer Immun.* **5**, 10 (2005).
18. S. Bauer *et al.*, *Arthritis Res. Ther.* **8**, R171 (2006).
19. Materials and methods are available as supporting material on Science Online.
20. A. Peister *et al.*, *Blood* **103**, 1662 (2004).
21. S. Bae *et al.*, *Br. J. Haematol.* **142**, 827 (2008).
22. R. Bucala, L. A. Spiegel, J. Chesney, M. Hogan, A. Cerami, *Mol. Med.* **1**, 71 (1994).
23. A. M. Santos, J. Jung, N. Aziz, J. L. Kissil, E. Puré, *J. Clin. Invest.* **119**, 3613 (2009).
24. D. J. Nelson *et al.*, *J. Immunol.* **166**, 5557 (2001).
25. J. Doukas, J. S. Pober, *J. Immunol.* **145**, 1727 (1990).
26. C. Ruegg *et al.*, *Nat. Med.* **4**, 408 (1998).
27. K. P. Olive *et al.*, *Science* **324**, 1457 (2009); 10.1126/science.1171362.
28. B. Zhang, T. Karrison, D. A. Rowley, H. Schreiber, *J. Clin. Invest.* **118**, 1398 (2008).
29. T. Schuler, T. Blankenstein, *J. Immunol.* **170**, 4427 (2003).
30. H. F. Dvorak, *N. Engl. J. Med.* **315**, 1650 (1986).
31. K. S. Peggs, S. A. Quezada, J. P. Allison, *Immunol. Rev.* **224**, 141 (2008).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6005/827/DC1

Materials and Methods

Figs. S1 to S9

Table S1

References

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Alleviating Cancer Drug Toxicity by Inhibiting a Bacterial Enzyme

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The dose-limiting side effect of the common colon cancer chemotherapeutic CPT-11 is severe diarrhea caused by symbiotic bacterial β -glucuronidases that reactivate the drug in the gut. We sought to target these enzymes without killing the commensal bacteria essential for human health. Potent bacterial β -glucuronidase inhibitors were identified by high-throughput screening and shown to have no effect on the orthologous mammalian enzyme. Crystal structures established that selectivity was based on a loop unique to bacterial β -glucuronidases. Inhibitors were highly effective against the enzyme target in living aerobic and anaerobic bacteria, but did not kill the bacteria or harm mammalian cells. Finally, oral administration of an inhibitor protected mice from CPT-11-induced toxicity. Thus, drugs may be designed to inhibit undesirable enzyme activities in essential microbial symbiotes to enhance chemotherapeutic efficacy.

Camptothecin, a potent antineoplastic compound, was added to the National Cancer Institute natural products screening set in 1966. It poisons the catalytic cycle of human topoisomerase I, which manages the superhelical tension associated with DNA metabolism and is preferentially active in rapidly dividing cells (1, 2). In preliminary clinical trials, camptothecin exhibited marked toxicity and poor bioavailability (3). Although its derivatives topotecan and CPT-11 (also called irinotecan) are now in clinical use (3), they still elicit pronounced side effects that limit efficacy. CPT-11 is one of the three commonly used chemotherapeutic agents for colon cancer, and it has also been used against lung and brain tumors as well as refractory forms of leukemia and lymphoma (4). It is a prodrug, with a carbamate-linked dipiperidino group that increases solubility and bioavailability (3); this moiety is removed in vivo to produce the active metabolite SN-38 (5) (Fig. 1A).

CPT-11 causes severe diarrhea generated by its complex activation and subsequent metabolism (Fig. 1A) (6, 7). SN-38 produced by carboxylesterases is glucuronidated in the liver by uridine diphosphate (UDP)-glucuronosyltransferase enzymes to form inactive SN-38G (8), which is excreted via the biliary ducts into the gastrointestinal (GI) tract (Fig. 1A). Once in the intestines, though, SN-38G serves as a substrate for bacterial β -glucuronidase enzymes in the commensal microbiota that remove the glucuronide group as a

carbon source, producing reactivated SN-38 in situ (Fig. 1A) (9, 10). SN-38 levels in the intestinal lumen play an essential role in the delayed diarrhea that prevents dose intensification and efficacy in up to 40% of treated patients (11–13).

The feasibility of using antibiotics to reduce GI bacteria levels prior to CPT-11 treatment has been examined (14); however, this approach has several drawbacks. Intestinal biota play essential roles in carbohydrate metabolism, vitamin production, and the processing of bile acids, sterols, and xenobiotics (15, 16). Thus, the removal of GI bacteria is not recommended for patients already challenged by neoplastic growths and chemotherapy. In addition, elimination of symbiotic GI flora increases the chances of infections by pathogenic bacteria, including enterohemorrhagic *Escherichia coli* and *Clostridium difficile* (17–23).

β -Glucuronidase enzymes hydrolyze glucuronic acid sugar moieties from a variety of compounds (24), and their presence in a range of bacteria is exploited to detect bacterial contamination in commonly used water purity tests (25). The crystal structure of human β -glucuronidase was reported in 1996 (26), but no structure of a bacterial β -glucuronidase has been presented. In addition, only relatively weak inhibitors of β -glucuronidases have been described [inhibition constant (K_i) values of 25 μ M to 2 mM] (27, 28). Thus, we used structural and chemical biology to identify potent and selective inhibitors of bacterial β -glucuronidases to eliminate the GI toxicity of CPT-11 without killing the bacterial symbiotes required for intestinal health.

Full-length *E. coli* β -glucuronidase was purified and shown to hydrolyze SN-38G to SN-38 in vitro (fig. S1). The enzyme was initially crystallized both alone and in complex with an established low-affinity inhibitor, glucaro- δ -lactam (GDL) (29), and data were collected to 2.5 and 2.4 Å resolution, respectively. Because molecular replacement using a previously reported human β -glucuronidase model [PDB ID 1BH9 (30)] was unsuccessful, selenomethionine (SeMet)-substituted *E. coli* β -glucuronidase and single-

wavelength anomalous dispersion x-ray data to 2.9 Å resolution were used for structure determination and refinement (PDB ID 3K4A). Molecular replacement using the SeMet model was then used to determine and refine the native (PDB ID 3K46), GDL-bound (PDB ID 3K4D), and Inhibitor 2 and Inhibitor 3 structures (PDB IDs 3LPF and 3LPG) (table S1).

The asymmetric unit of the *E. coli* β -glucuronidase structure contains two monomers of 597 ordered residues, and crystallographic symmetry generates the functionally relevant enzyme tetramer observed previously for the human enzyme (30) and confirmed by gel filtration chromatography for the *E. coli* form of the enzyme (Fig. 1B). The N-terminal 180 residues resemble the sugar-binding domain of family 2 glycosyl hydrolases (31), whereas the C-terminal domain (residues 274 to 603) forms an $\alpha\beta$ barrel (31) and contains the active-site residues Glu⁴¹³ and Glu⁵⁰⁴. The region between the N- and C-terminal domains exhibits an immunoglobulin-like β -sandwich domain consistent with other family 2 glycosyl hydrolases (31, 32) (fig. S2). The GDL inhibitor binds in a single orientation deep within the active site of the enzyme's C-terminal domain and involves large (>14 Å) shifts in residue positions relative to the unliganded structure (figs. S3 and S4). Superimposing the *E. coli* β -glucuronidase structure on the human enzyme reveals a 1.4 Å root mean square deviation over 565 equivalent C α positions with 45% sequence identity (figs. S5 and S6). The *E. coli* enzyme contains a 17-residue "bacterial loop" not found in the human ortholog, with the active site of each *E. coli* β -glucuronidase monomer containing "bacterial loops" from that monomer as well as a neighboring monomer within the enzyme tetramer (fig. S4).

Chemical library screening was conducted using a β -glucuronidase assay in which the conversion of 4-methylumbelliferyl-glucuronide (4-MUG) to 4-methylumbelliferone (4-MU) was monitored by measuring the increase in 4-MU fluorescence (excitation at 365 nm, emission at 450 nm) (fig. S7) (25). This assay exhibited robust characteristics when used with a 10,240-compound chemical library, with a screening Z-factor of 0.84 (33). The hit rate was 1%, with 100 compounds producing 90% inhibition or better and R^2 values for inhibition curves of 0.99 or better. Four compounds were chosen for further investigation (Fig. 1C). Secondary β -glucuronidase assays were also used to examine inhibitor potency in vitro and in cell-based studies.

The GDL compound exhibited relatively weak in vitro enzyme inhibition [e.g., median inhibitory concentration (IC_{50}) = 26.4 ± 4.83 μ M] and did not disrupt β -glucuronidase activity in cultured *E. coli* cells (fig. S8). In contrast, the four compounds chosen from the high-throughput screen (Fig. 1C) were all potent in vitro inhibitors with submicromolar IC_{50} and K_i values and uncompetitive characteristics as assessed by their lack of impact on enzyme k_{cat}/K_m values (Table 1 and fig.

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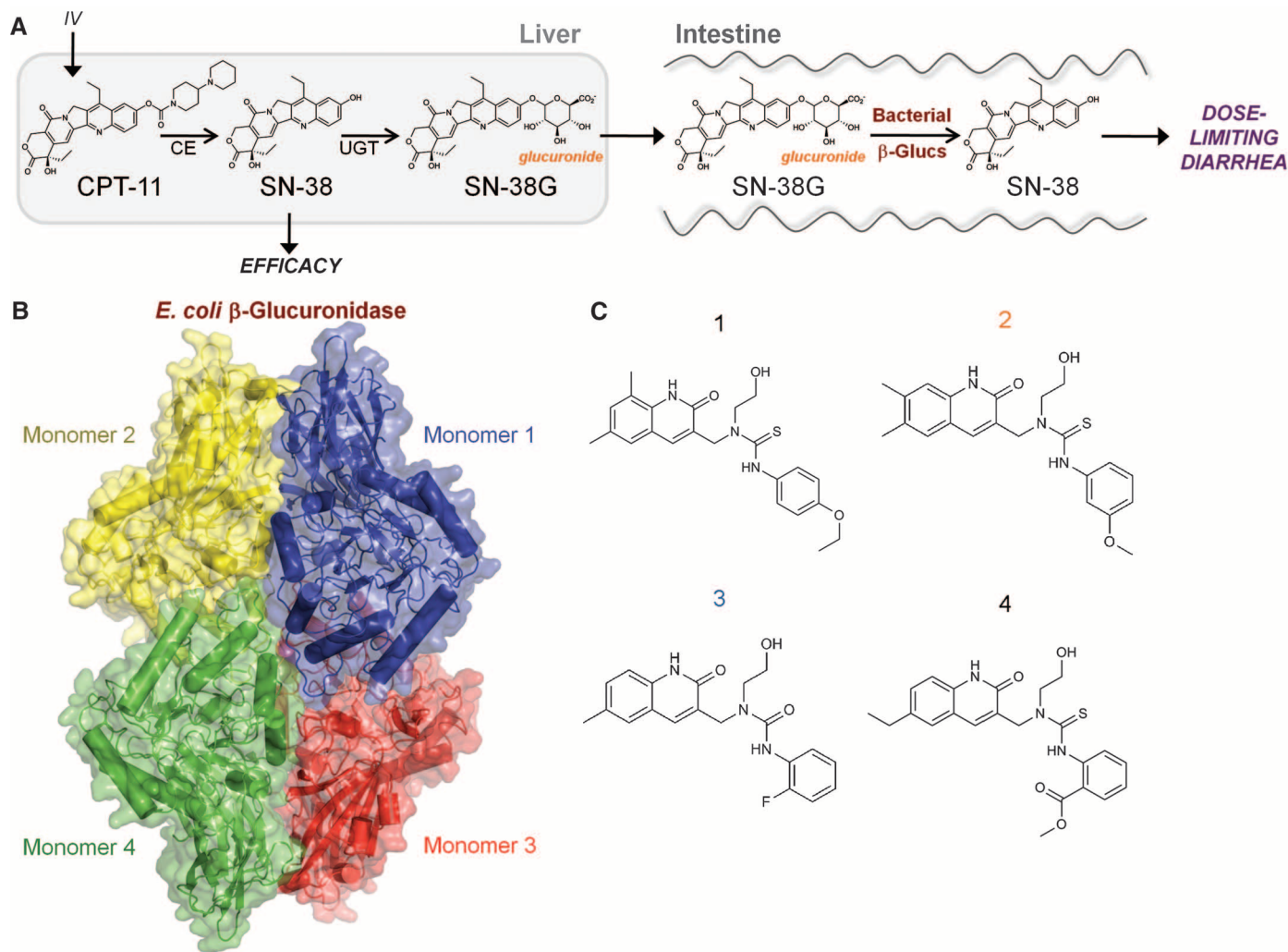


Fig. 1. CPT-11 metabolism and *E. coli* β-glucuronidase. **(A)** Intravenously administered CPT-11 is activated by carboxylesterases (CE) to SN-38, an antineoplastic topoisomerase I poison. Liver SN-38 is inactivated via glucuronidation to SN-38G by UDP-glucuronosyltransferase (UGT) enzymes and sent to the intestines. β-Glucuronidases (β-glucs) in the symbiotic

GI bacteria remove the glucuronide as a carbon source, and active SN-38 in the intestinal lumen generates dose-limiting diarrhea. **(B)** Crystal structure of the *E. coli* β-glucuronidase tetramer at 2.5 Å resolution. **(C)** Four selective bacterial β-glucuronidase inhibitors identified via high-throughput screening.

S9). Crystal structures of *E. coli* β-glucuronidase in complexes with Inhibitors 2 and 3 were resolved to 2.3 and 2.4 Å resolution, respectively (table S1), and revealed that the inhibitors bound at the “bacterial loops” at the entrance to the active-site cavity, with the ethoxy groups extending to within 3.3 Å of the catalytic Glu⁴¹³ residue (Fig. 2A). Primary contacts between the enzyme monomer and inhibitors involved the “bacterial loop” (Leu³⁶¹) of the primary monomer, as well as the “bacterial loop” (Phe³⁶⁵) of an adjacent monomer within the tetramer (Fig. 2B and fig. S10). Thus, this loop may be essential to inhibitor efficacy.

The specificity of the four characterized inhibitors for bacterial versus mammalian β-glucuronidases was assayed in vitro with the use of bovine liver β-glucuronidase. With a concentration range of 0 to 100 μM for each of the inhibitors, no effect was observed on the activity of this mammalian β-glucuronidase (figs. S11

Table 1. In vitro and bacterial cell-based assays for β-glucuronidase activity and inhibitor efficacy. k_{cat} , catalytic rate; K_m , Michaelis constant; N/A, not applicable. N.E., not effective. Errors represent standard deviation, where $N = 3$.

	<i>E. coli</i> β-glucuronidase in vitro			<i>E. coli</i> cell-based
	IC ₅₀ (nM)	k_{cat}/K_m (s ^{−1} μM ^{−1})	K _i (nM)	EC ₅₀ (nM)
No inhibitor	N/A	0.134 ± 0.0123	N/A	N/A
Glucaro-δ-lactam	26400 ± 483	N/A	7750 ± 475	N.E.
Inhibitor 1	283 ± 26.1	0.0987 ± 0.00621	164 ± 13.0	17.7 ± 7.42
Inhibitor 2	369 ± 2.51	0.119 ± 0.00473	208 ± 25.4	28.3 ± 2.11
Inhibitor 3	586 ± 31.1	0.136 ± 0.00633	684 ± 81.6	233.2 ± 2.99
Inhibitor 4	1060 ± 3.54	0.122 ± 0.0205	1380 ± 166	1322.8 ± 1.15

and S12). The 17-residue loop that contacts the inhibitors in the crystal structures of *E. coli* β-glucuronidase (Fig. 2, A and B) is not present in mammalian forms of the enzyme (fig. S13), which act on larger substrates such as glycosaminoglycans (34). Of the 284 GI-associated bacterial species in the Human Microbiome Project database

(35), 123 (43%) contained β-glucuronidases or candidate β-glucuronidases; of those, 121 (98%) maintained at least the N-terminal portion of the “bacterial loop,” and 110 (91%) contained the key residues at *E. coli* β-glucuronidase positions 361 and 365 capable of making the inhibitor contacts reported here (fig. S14; see also figs. S10

Fig. 2. Potent β -glucuronidase inhibitors. (A) Crystal structures of Inhibitors 2 and 3 bound to the active site of *E. coli* β -glucuronidase. (B) Inhibitors are observed to stack co-operatively between monomers in the *E. coli* β -glucuronidase tetramer. Amino acid abbreviations: D, Asp; E, Glu; F, Phe; G, Gly; L, Leu; M, Met; R, Arg; S, Ser; Y, Tyr.

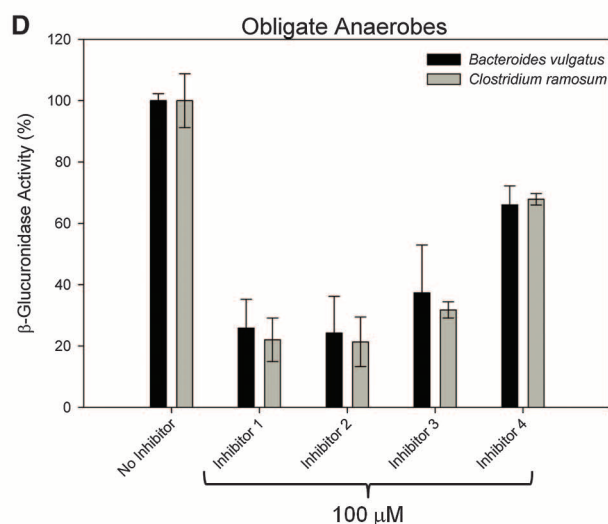
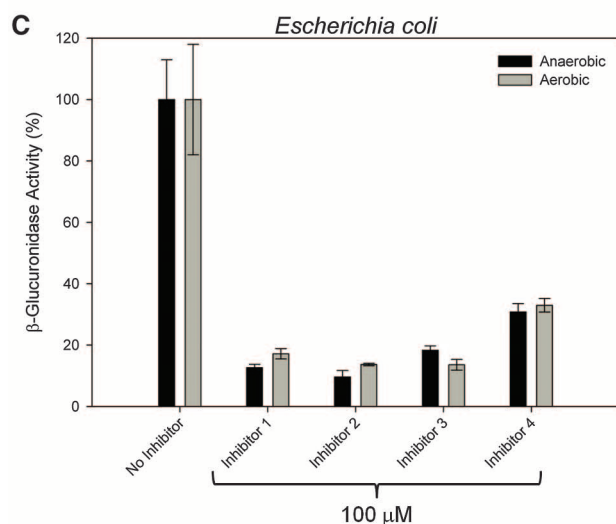
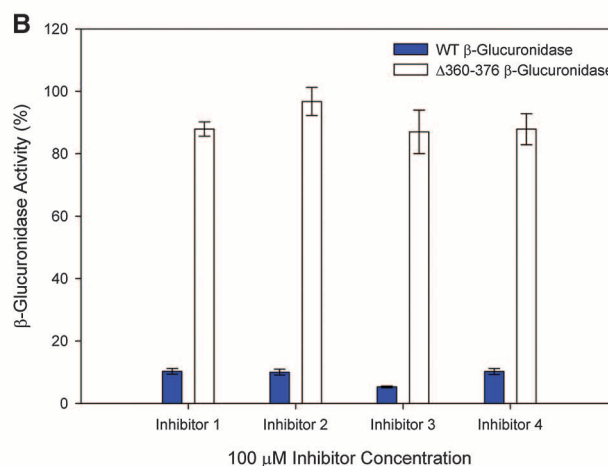
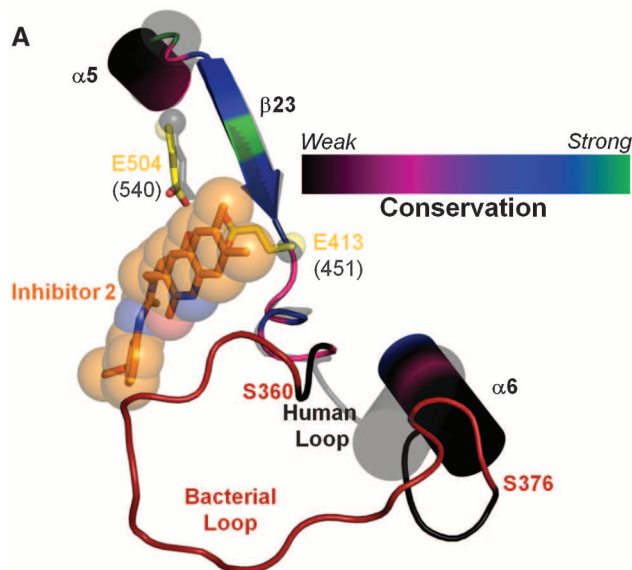
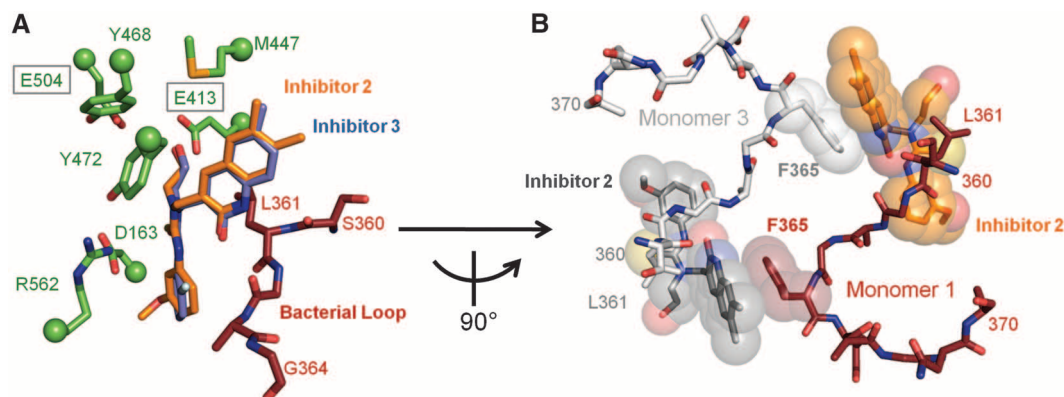


Fig. 3. Inhibitor selectivity for bacterial β -glucuronidase. (A) The 360–376 loop forms direct contact with the bound inhibitors in the *E. coli* β -glucuronidase structure. This loop is missing from the structure of human β -glucuronidase; thus, it is labeled the “bacterial loop.” (B) Elimination of the

“bacterial loop” from *E. coli* β -glucuronidase produces an enzyme insensitive to inhibitor efficacy. (C) β -Glucuronidase inhibition in living *E. coli* cells grown under both aerobic and anaerobic conditions. (D) β -Glucuronidase inhibition in two obligate anaerobic bacteria. Error bars represent SD; $N = 3$.

and S13 and table S2). To test the hypothesis that the “bacterial loop” is required for the efficacy of our inhibitors, we created a form of *E. coli* β -

glucuronidase that lacks residues 360 to 376 (Fig. 3A). The Δ 360-376 *E. coli* β -glucuronidase mutant was not inhibited even at 100 μ M concen-

trations of the four compounds examined (Fig. 3B). Thus, we have identified inhibitors that are potent and selective for a bacterial β -glucuronidase.

We next examined the ability of the four lead compounds to inhibit β -glucuronidase activity in living bacterial cells. Whereas a $\sim 26 \mu\text{M}$ in vitro inhibitor such as GDL has no effect against β -glucuronidase activity in cultured *E. coli*, each inhibitor tested was effective, with median effective concentration (EC_{50}) values from 18 nM to 1.3 μM (Table 1 and fig. S15). Because >99% of the microbial species present in the GI tract are obligate anaerobes (36), we tested *E. coli* cells grown under anaerobic conditions and examined other relevant anaerobic bacterial species (35). The compounds were effective in *E. coli* grown under both aerobic and anaerobic conditions (Fig. 3C) and were effective against enzyme activity in two obligate anaerobes known to inhabit the mammalian GI, *Bacteroides vulgatus* and *Clostridium ramosum* (Fig. 3D). We also tested *Lactobacillus reuteri* and *Bifidobacterium infantis*, which do not contain the gene encoding β -glucuronidase (28, 37), and found no evidence of enzyme activity or inhibitor impact on cell viability for these or other strains (figs. S16 and S17). Consistent

with their lack of effect on mammalian β -glucuronidase activity in vitro, inhibitors at relatively high concentrations (100 μM) had little to no effect on the survival of two human colon cancer cell lines (HCT116, Caco-2) and one murine colon cancer cell line (CMT93) (38) (fig. S18).

Finally, we examined the ability of Inhibitor 1 to eliminate the delayed diarrhea and intestinal damage caused by CPT-11 administration. Healthy 6- to 8-week-old Balb/cJ mice were divided into four groups of 16 animals each. Group 1 received 50 μl of double-distilled H_2O intraperitoneally (i.p.) and 1% dimethyl sulfoxide by oral gavage twice daily. Group 2 received 10 μg of Inhibitor 1 via oral gavage twice per day, but no CPT-11. Group 3 received CPT-11 i.p. once daily, at a dose of 50 mg per kg of body weight, and no Inhibitor 1. Group 4 received CPT-11 and Inhibitor 1 with dose and schedule identical to animals in group 3 and group 2, respectively. GI symptoms appeared after 7 days. At days 8 to 10, all the animals on the CPT-11-only treatment (group 3) experienced both diarrhea and

bloody diarrhea, whereas none of the animals receiving vehicle or inhibitor alone (groups 1 and 2, respectively) exhibited diarrhea at any point during the study (Fig. 4A and fig. S19). By day 11, all the group 3 animals had to be euthanized. In contrast, the animals receiving both CPT-11 and Inhibitor 1 (group 4), exhibited less diarrhea and bloody diarrhea than did the group 3 animals (Fig. 4A and fig. S19). Examination and scoring of colonic tissue samples from each group established that Inhibitor 1 protected the mouse GI epithelium from CPT-11-induced damage (Fig. 4, B and C) (39). Animals treated with vehicle and Inhibitor 1 exhibited healthy glandular structure and an intact epithelial layer; CPT-11 administration destroyed these tissues, eliminating the glands and causing a large influx of inflammatory cells (Fig. 4, B and C). In contrast, Inhibitor 1, when provided orally in combination with i.v. CPT-11, protected the glandular structure of these intestinal tissues (Fig. 4, B and C).

The lead compounds that we have characterized from high-throughput screening hits are

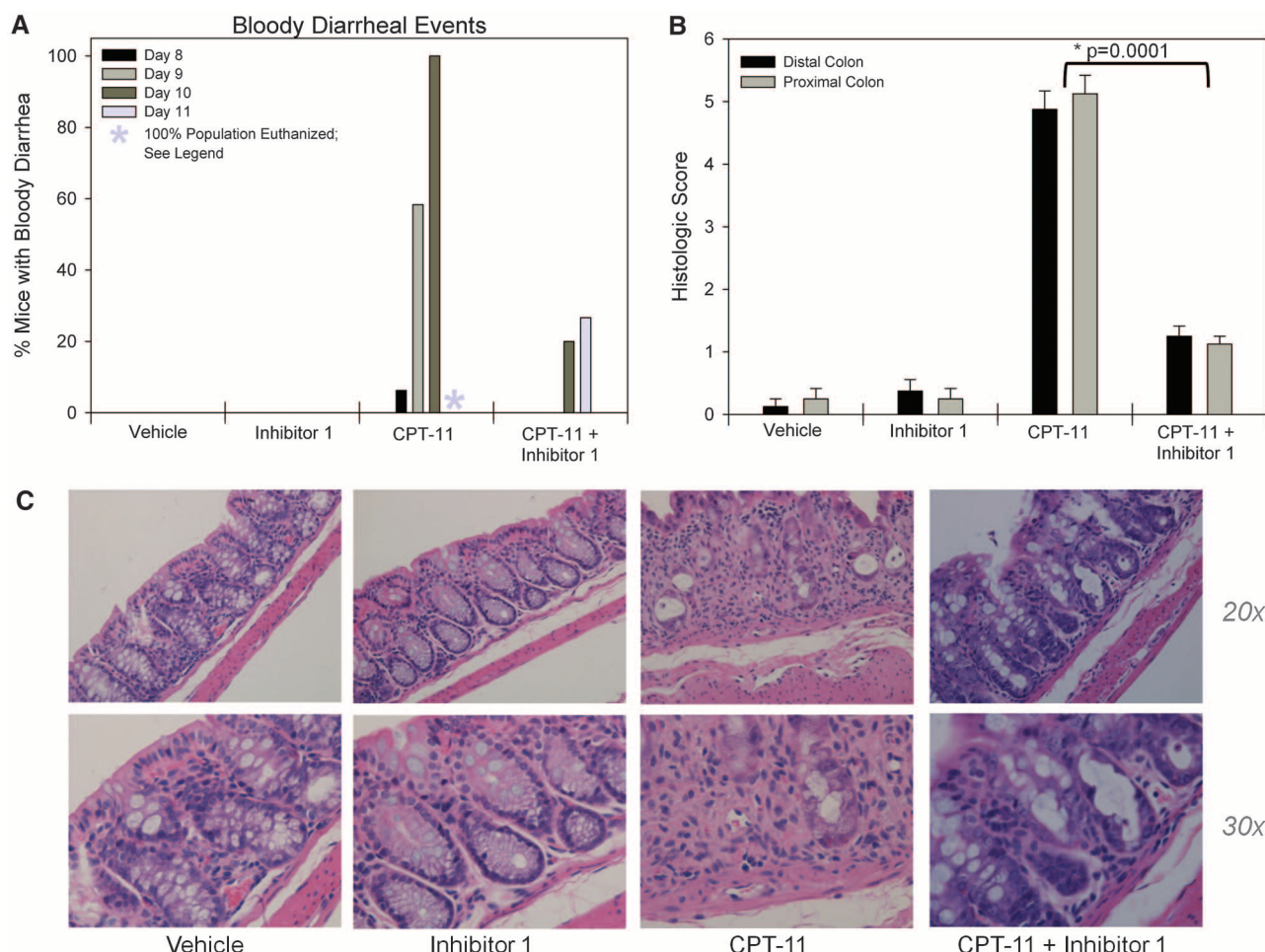


Fig. 4. Alleviation of CPT-11 toxicity in mice. (A) CPT-11 produced bloody diarrhea starting after 8 days and peaking at 10 days, whereas oral administration of Inhibitor 1 with CPT-11 reduced the incidence of bloody diarrhea. Vehicle and Inhibitor 1 alone caused no bloody diarrhea. By day 8 to 11, mice in the CPT-11 group began to suffer from severe lethargy and lack of movement; by day 11, all mice in that group were euthanized according to AIC

protocol 20070715. (B) Histologic score of the distal and proximal colon of animals in the four treatment groups. Error bars represent SD; $N = 12$. (C) Tissue histology of colons taken from mice from each treatment group show healthy glandular structure for both vehicle and Inhibitor 1 but highly disrupted tissues in the CPT-11 group. In contrast, Inhibitor 1 provided in combination with CPT-11 protects the colon from damage.

all effective inhibitors of the β -glucuronidase enzyme in vitro (with K_i values of 160, 210, 680, and 1400 nM, respectively), and they maintain potent efficacy in living bacterial cells (EC_{50} values of 18, 28, 230, and 1300 nM, respectively) (Table 1) without affecting bacterial cell growth or survival under aerobic or anaerobic conditions (figs. S16 and S17) or killing mammalian epithelial cells (fig. S18). Lower EC_{50} values relative to the K_i values likely reflect relatively low β -glucuronidase concentrations in cells. Key regions of the “bacterial loop” identified in the *E. coli* β -glucuronidase crystal structure are present in 98% of the β -glucuronidases sequenced from human GI bacteria, and 91% of those sequences contain residues that appear capable of forming inhibitor contacts (figs. S13 and S14). Disruption of β -glucuronidase activity is also demonstrated in bacterial species beyond *E. coli* (Fig. 3D). Taken together, the data presented here strongly support the hypothesis that microbial β -glucuronidases can be inhibited to prevent the GI production of toxic CPT-11 metabolites. However, full pharmacokinetic studies demonstrating, for example, a reduction in GI SN-38G levels or improved CPT-11 efficacy will be required to unambiguously prove this hypothesis. In addition, the breadth of inhibitor efficacy on human GI bacteria requires further assessment. Still, these initial results involving the oral dosing of an unmodified lead compound are highly promising. If successfully translated to humans, leads like those described here could allow dose intensification of CPT-11, enabling studies of whether efficacy could be improved in relevant human cancers by reducing one of the current dose-limiting side effects. The strategy of selective targeting of an enzyme present in bacterial symbiotes to address a specific clinical problem could po-

tentially be applied more broadly as we deepen our understanding of the essential and dynamic roles that commensal bacteria play in promoting human health.

References and Notes

- Y. H. Hsiang, R. Hertzberg, S. Hecht, L. F. Liu, *J. Biol. Chem.* **260**, 14873 (1985).
- M. R. Redinbo, J. J. Champoux, W. G. Hol, *Curr. Opin. Struct. Biol.* **9**, 29 (1999).
- J. F. Pizzolato, L. B. Saltz, *Lancet* **361**, 2235 (2003).
- Y. Pommier, *Nat. Rev. Cancer* **6**, 789 (2006).
- N. F. Smith, W. D. Figg, A. Sparreboom, *Toxicol. In Vitro* **20**, 163 (2006).
- R. H. J. Mathijssen *et al.*, *Clin. Cancer Res.* **7**, 2182 (2001).
- M. K. Ma, H. L. McLeod, *Curr. Med. Chem.* **10**, 41 (2003).
- S. Nagar, R. L. Blanchard, *Drug Metab. Rev.* **38**, 393 (2006).
- P. J. Tobin, H. M. Dodds, S. Clarke, M. Schnitzler, L. P. Rivory, *Oncol. Rep.* **10**, 1977 (2003).
- A. Stein, W. Voigt, K. Jordan, *Ther. Adv. Med. Oncol.* **2**, 51 (2010).
- A. Kurita *et al.*, *Cancer Chemother. Pharmacol.* **10.1007/s00280-010-1310-4** (2010).
- Z. P. Hu *et al.*, *Toxicol. Appl. Pharmacol.* **216**, 225 (2006).
- See supporting material on Science Online.
- D. Flieger *et al.*, *Oncology* **72**, 10 (2007).
- J. H. Cummings, G. T. Macfarlane, *J. Parenter. Enteral Nutr.* **21**, 357 (1997).
- F. Guarner, J. R. Malagelada, *Lancet* **361**, 512 (2003).
- S. B. Levy, B. Marshall, *Nat. Med.* **10** (suppl.), S122 (2004).
- C. E. Nord, L. Kager, A. Heimdahl, *Am. J. Med.* **76**, 99 (1984).
- C. D. Settle, M. H. Wilcox, *Aliment. Pharmacol. Ther.* **10**, 835 (1996).
- S. Sears, P. McNally, M. S. Bachinski, R. Avery, *Gastrointest. Endosc.* **50**, 841 (1999).
- D. Stamp, *Med. Hypotheses* **63**, 555 (2004).
- L. Yang, Z. Pei, *World J. Gastroenterol.* **12**, 6741 (2006).
- S. H. Cohen *et al.*, *Infect. Control Hosp. Epidemiol.* **31**, 431 (2010).
- A. Basińska, B. Floriańczyk, *Ann. Univ. Mariae Curie Skłodowska Med.* **58**, 386 (2003).
- A. H. Farnleitner, L. Hocke, C. Beiwil, G. G. Kavka, R. L. Mach, *Water Res.* **36**, 975 (2002).
- S. Jain *et al.*, *Nat. Struct. Biol.* **3**, 375 (1996).
- M. Fittkau, W. Voigt, H.-J. Holzhausen, H. J. Schmoll, *J. Cancer Res. Clin. Oncol.* **130**, 388 (2004).
- W. M. Russell, T. R. Klaenhammer, *Appl. Environ. Microbiol.* **67**, 1253 (2001).
- T. Niwa, T. Tsuruoka, S. Inoue, Y. Naito, T. Koeda, *J. Biochem.* **72**, 207 (1972).
- S. Jain *et al.*, *Nat. Struct. Mol. Biol.* **3**, 375 (1996).
- R. H. Jacobson, X. J. Zhang, R. F. DuBose, B. W. Matthews, *Nature* **369**, 761 (1994).
- A. Marchler-Bauer *et al.*, *Nucleic Acids Res.* **37** (database issue), D205 (2009).
- J. H. Zhang, T. D. Chung, K. R. Oldenburg, *J. Biomol. Screen.* **4**, 67 (1999).
- J. Ray, V. Scarpino, C. Laing, M. E. Haskins, *J. Hered.* **90**, 119 (1999).
- P. J. Turnbaugh *et al.*, *Nature* **449**, 804 (2007).
- C. L. Sears, *Anaerobe* **11**, 247 (2005).
- J. P. Grill, C. Manginot-Dürr, F. Schneider, J. Ballongue, *Curr. Microbiol.* **31**, 23 (1995).
- M. G. Brattain, W. D. Fine, F. M. Khaled, J. Thompson, D. E. Brattain, *Cancer Res.* **41**, 1751 (1981).
- B. Siegmund *et al.*, *J. Pharmacol. Exp. Ther.* **296**, 99 (2001).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6005/831/DC1
Materials and Methods
SOM Text
Figs. S1 to S19
Tables S1 and S2
References

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The Mechanism for Activation of GTP Hydrolysis on the Ribosome

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Protein synthesis requires several guanosine triphosphatase (GTPase) factors, including elongation factor Tu (EF-Tu), which delivers aminoacyl-transfer RNAs (tRNAs) to the ribosome. To understand how the ribosome triggers GTP hydrolysis in translational GTPases, we have determined the crystal structure of EF-Tu and aminoacyl-tRNA bound to the ribosome with a GTP analog, to 3.2 angstrom resolution. EF-Tu is in its active conformation, the switch I loop is ordered, and the catalytic histidine is coordinating the nucleophilic water in position for inline attack on the γ -phosphate of GTP. This activated conformation is due to a critical and conserved interaction of the histidine with A2662 of the sarcin-ricin loop of the 23S ribosomal RNA. The structure suggests a universal mechanism for GTPase activation and hydrolysis in translational GTPases on the ribosome.

In all stages of protein synthesis the ribosome requires exogenous protein factors, including several guanosine 5'-triphosphate (GTP)-hydrolyzing enzymes known as GTPases. These proteins are essential and highly conserved and include elongation factor Tu (EF-Tu), which de-

livers aminoacyl-tRNA to the ribosome as part of a ternary complex (TC) with GTP, as well as elongation factor G and initiation factor 2 (I). The architecture of the GTP-binding domain is similar in all translational GTPases from bacteria to higher eukaryotes, and hydrolysis is accom-

panied by conformational changes in the conserved switch I and II regions (2). Furthermore, translational GTPases bind to the same region of the ribosome in all species (3, 4). The conserved binding site and structural similarities suggest that there is a common mechanism by which the ribosome activates GTP hydrolysis in these factors. However, despite more than 40 years of research, this mechanism has remained elusive.

The catalysis of GTP hydrolysis in translational GTPases requires an invariant histidine (His⁸⁴ in EF-Tu) that acts as a general base, abstracting a proton from a water molecule, for inline attack on the γ -phosphate of GTP (5–7). The intrinsic GTPase activity of translational GTPases

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is low (8) and increases markedly upon productive binding to the ribosome (9, 10). For EF-Tu, efficient GTPase activation requires the binding of an aminoacyl-tRNA to its cognate mRNA codon. Activation could be accomplished either indirectly, i.e., proper ribosome binding induces an active conformation of the G factor, and/or more directly, with the ribosome playing an active role in catalysis. The ribosomal components that compose the GTPase binding site and therefore may be important for hydrolysis include the sarcin-ricin loop (SRL) of the 23S ribosomal RNA (rRNA) (3, 11), the L11 protein and rRNA (12), and the protein L12 (13).

Premature GTP hydrolysis in EF-Tu is thought to be prevented by a “hydrophobic gate,” consisting of residues Val²⁰ of the P loop and Ile⁶⁰ of switch I, which restricts access of His⁸⁴ to the catalytic water (6). Proposals of how the hydrophobic gate is “opened” include that His⁸⁴ would simply “push” through the gate when ac-

tivated (6), or that a disordering (14, 15) or radical rearrangement of switch I removes this steric block (16–18). However, these hypotheses were made on the basis of structures of EF-Tu in isolation, or studies of the posthydrolysis, kirromycin-stalled complex on the ribosome. A conclusive understanding of how GTPase activation occurs on the ribosome requires the structure of a complex of EF-Tu before GTP hydrolysis.

Here, we report the crystal structure of EF-Tu bound to the 70S ribosome along with Trp-tRNA^{Trp}, and the antibiotic paromomycin, stalled in the activated conformation by the GTP analog β - γ -methylene-5'-triphosphate (GDP-CP), at 3.2 Å resolution. The structure reveals the GTPase-competent form of EF-Tu and suggests a universal mechanism for GTP hydrolysis on the ribosome.

The overall conformations of the ribosome, EF-Tu, and the aminoacyl-tRNA are as previously reported (15) (Fig. 1A), and all the conformational changes predicted to communicate codon recog-

nition to EF-Tu are observed, except that the switch I loop is ordered in the GTPase center (Fig. 1B). Additionally, the catalytic histidine is activated and coordinating a water for inline attack on the γ -phosphate of the GTP analog (Fig. 1C).

As well as providing insight into the mechanism of GTP hydrolysis of all translational GTPases, this structure represents a key, missing state in the decoding pathway. High-resolution crystal structures have previously been determined for the isolated TC (19) and the posthydrolysis state stalled on the ribosome by kirromycin (15). In conjunction with these two structures, this structure of the activated GTP state bound to the ribosome provides insight into the chemical details of the complete hydrolysis pathway of EF-Tu.

Before ribosome binding, the TC contains an unbound tRNA, and switch I, switch II, and the P loop are ordered around the GTP molecule in the active site. However, the catalytic His⁸⁴ (switch II) is rotated away from GTP in an inactive con-

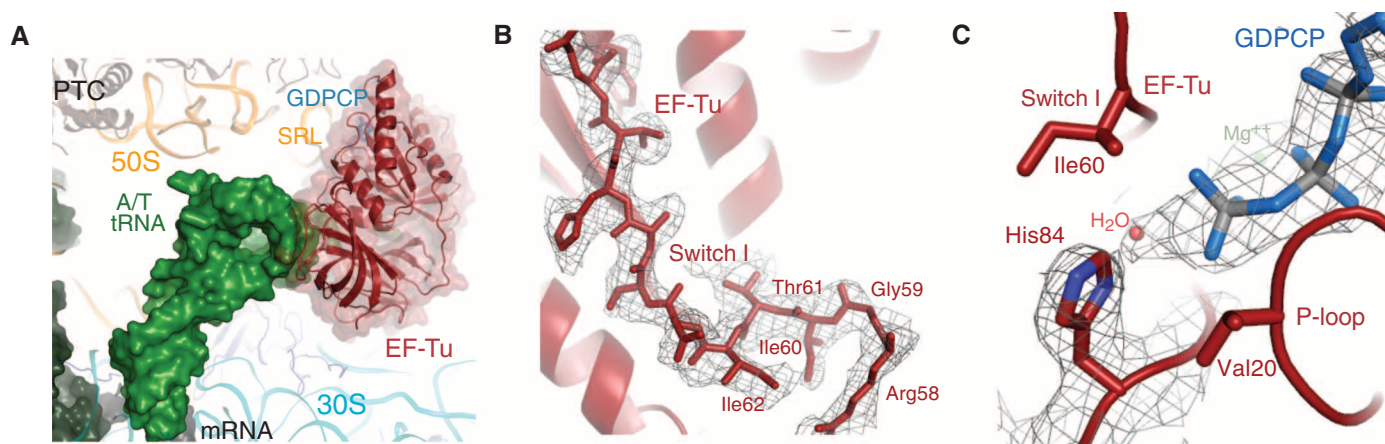


Fig. 1. The ternary complex bound to the ribosome in the activated state. (A) EF-Tu (red) and aminoacyl-tRNA (green) bound to the 70S ribosome, stabilized by GDP-CP (blue). (B) Unbiased $F_o - F_c$ electron density displayed for the switch

I loop. (C) Unbiased $F_o - F_c$ electron density for GDP-CP, the water molecule (red sphere), and His⁸⁴ positioned in a catalytically active conformation. *E. coli* number is used throughout the text.

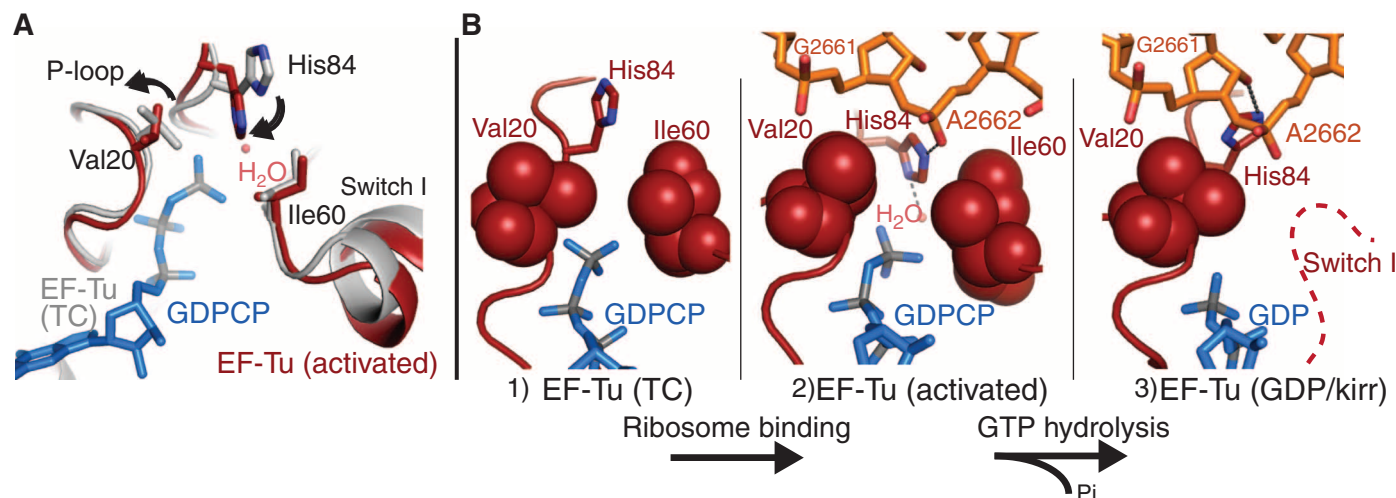


Fig. 2. The active site of EF-Tu during decoding. (A) Superposition of the GTPase centers from the isolated TC (gray) (19) and the TC-GDP-CP-ribosome (red). Small movements are observed for hydrophobic gate residues Val²⁰ and

Ile⁶⁰. (B) GTPase activation allows the phosphate of A2662 of the SRL (orange) to position His⁸⁴ into the active site. After GTP hydrolysis (15) and P_i release, switch I becomes disordered (dashed line) and His⁸⁴ rotates away from GDP.

formation (19). Ribosome binding and codon recognition induce the bent tRNA in the A/T state (where the anticodon interacts with the mRNA in the A site and the acceptor end is bound to EF-Tu) (20), as well as conformational changes in the tRNA, 30S ribosomal subunit, and EF-Tu essential for triggering GTP hydrolysis (15). Productive binding of the TC to the ribosome results in a shift of the G domain of EF-Tu by up to ~ 7 Å, to avoid a steric clash between switch I and the SRL of the 23S rRNA. Ribosome binding also causes a distortion in the 3' end of the aminoacyl-tRNA between residues 72 and 75 as reported (15), which releases contacts between the tRNA backbone and the switch I loop (fig. S1). However, in contrast to our previous prediction, this alone does not disorder switch I, which is largely unchanged from its conformation in the isolated TC (Fig. 2A) (19).

Although switch I remains ordered in the active site, the catalytic histidine is in its activated conformation (Fig. 2). We see no evidence that large rearrangements are required for catalysis (fig. S1) (15–18). Instead, the “open” state is reached by a 1.2 Å shift in the side chain of Ile⁶⁰, an $\sim 80^\circ$ rotation of the side chain of Val²⁰, and a small movement of the backbone of residues 19 to 22 away from the GTP analog (Fig. 2). The conformation of switch I is similar to that modeled for the eukaryotic ortholog of EF-G bound to β - γ -imidoguanosine 5'-triphosphate (GDPNP) on the ribosome (21).

These small changes in the active site would subtly increase the space between switch I and the P loop, possibly facilitating GTP hydrolysis. However, our analysis suggests that even the “closed” hydrophobic gate (19) could accommodate the activated conformation of His⁸⁴ and would not preclude binding of a water in its catalytic

position, though a water is not observed. Instead, to prevent GTP hydrolysis before codon recognition, the activated conformation of His⁸⁴ must be unfavorable in solution. Indeed, the adjacent residues, Gly⁸³ and Pro⁸², are more than 98% conserved, and mutational data suggest that the flexibility of these residues may be important (19, 22).

GTPase activation by the ribosome therefore does not involve an opening of the hydrophobic gate, but rather requires the specific positioning of His⁸⁴ into the active site. In this activated structure of EF-Tu, the phosphate of residue A2662 of the SRL orders His⁸⁴ in its catalytic conformation, which then acts as a general base to allow the nucleophilic water molecule to attack the γ -phosphate of GTP (Figs. 2 and 3). When positioning His⁸⁴, the phosphate of A2662 could also conceivably participate in a charge-relay system that facilitates the deprotonation of the water by His⁸⁴, playing a role analogous to that of the catalytic aspartic acid in serine proteases (23). However, the existence and contribution of such a mechanism would need to be assessed by biochemical experiments. The activation does not require movements within the SRL, as its overall conformation is similar to that in previous 70S ribosome structures (15, 24). This critical role of the sarcin-ricin loop answers the long-standing question of why the SRL is important for binding and GTPase activation by the ribosome. The toxin α -sarcin acts by cleaving the 23S rRNA between residues G2661 and A2662 (11), exactly the phosphate group activating His⁸⁴. It is therefore clear why cleaving this bond, or introducing mutations at nearby sites in the SRL (25), leads to defects in His⁸⁴ positioning and GTP hydrolysis (11, 26). α -Sarcin is active against both bacterial and eukaryotic ribosomes and causes similar defects in the function of their respective

translational GTPases (11, 26). We therefore predict that the positioning of the catalytic histidine into the active site by A2662 of the SRL is the universal mechanism for GTPase activation on the ribosome. EF-G has additional interactions between its domain 3 and A2660 of the SRL, which explains why depurination of A2660 by the toxin ricin affects EF-G, but not EF-Tu (26).

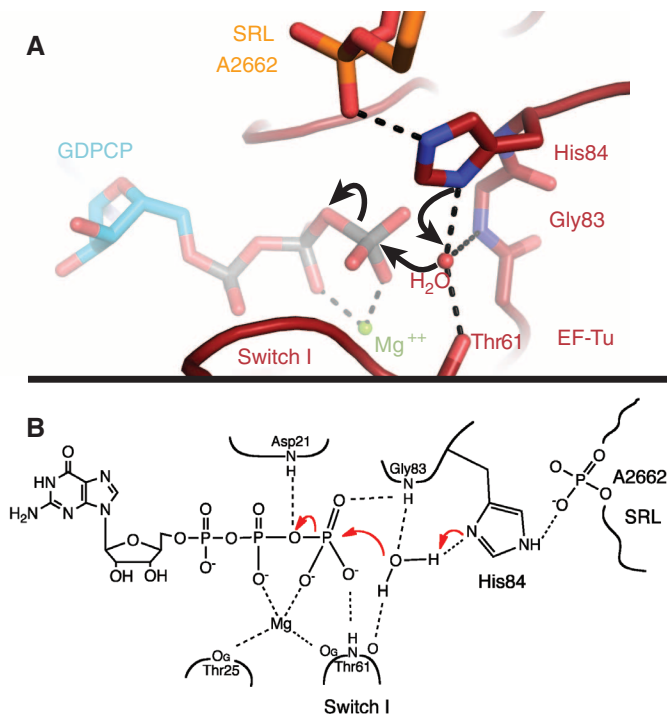
Ribosome association alone is not sufficient for A2662 to activate His⁸⁴. Rather, all of the conformational changes that occur upon cognate codon recognition in the ribosome (codon-anticodon monitoring, domain closure), the tRNA (A/T state, 3' end distortion), and EF-Tu (β -loop interaction with 30S shoulder, G domain shift) (15) are essential for properly positioning the GTPase center of EF-Tu for activation by the SRL. The energy required to induce these movements is balanced against the energy derived from binding of a cognate tRNA, including that from interactions of the 16S rRNA residues A1492, A1493, and G530 with the minor groove of the codon-anticodon helix (27). Even subtle changes in the position of the G domain relative to the SRL would cause defects in GTPase activation by preventing A2662 from properly placing His⁸⁴ into the active site.

The activation of GTP hydrolysis in EF-Tu by the SRL is similar to the regulation of cellular GTPases by their partner proteins. Unlike the classic cases of Rho and Ras, in which their GTPase activator proteins (GAPs) directly donate catalytic residues (28, 29), other G proteins are activated by “regulator of G-protein signaling” (RGS) proteins, which instead stabilize the active conformation of the GTPase (30). By analogy, the SRL acts as an RGS-type GAP for translational GTPases.

All components required for GTPase activation should be present in this catalytically active structure. Notably, we see no evidence that ribosomal protein L12, predicted to be important for hydrolysis, is interacting with EF-Tu, though such an interaction would be compatible with the crystal packing. Indeed, it is sterically impossible for L12 or any other protein to interact with GTP, consistent with data suggesting that L12 does not function directly in catalysis (13, 31, 32). Furthermore, deletion of the eukaryotic orthologs of L12 from yeast ribosomes is not lethal (33), indicating that L12 may not be critical for achieving GTP hydrolysis.

Following hydrolysis, inorganic phosphate (P_i) is released from EF-Tu. The γ -phosphate of GTP makes several contacts with switch I through residue Thr⁶² (Fig. 3 and fig. S3), and release of P_i could destabilize and perhaps even require movement of switch I. This and the loss of interactions with the 3' end of the tRNA backbone (fig. S1) lead to the previously observed disordering of switch I (15, 18). Furthermore, comparison of the kirromycin- and the GDCP-stalled complexes shows a rotation of $\sim 4^\circ$ of the G domain relative to domains 2 and 3 (fig. S4). There is a corresponding movement in the acceptor stem of the tRNA, in order to maintain interactions with switch II (15), which could explain the subtle

Fig. 3. Chemical mechanism of GTP hydrolysis. **(A)** Highly conserved His⁸⁴ acts as a general base to activate the catalytic water molecule, which is positioned by interactions with Thr⁶¹, Gly⁸³, and His⁸⁴. **(B)** Chemical structure diagram depicting the interactions between EF-Tu and GTP that stabilize the β and γ phosphates, allowing GTP hydrolysis to occur (see also fig. S3).



difference in fluorescence signal between the activated and kirromycin-installed states (34).

This structure of 70S-rRNA-EF-Tu-GDPCP provides an atomic-resolution model of a translational GTPase in its activated state. Codon recognition leads to a series of conformational changes in the 30S subunit, tRNA, and EF-Tu that position EF-Tu for GTP hydrolysis. GTPase activation does not require a large opening of the “hydrophobic gate,” but instead requires the positioning of the catalytic histidine into the active site by the SRL residue A2662. The high level of sequence and structural conservation between all translational GTPases suggests that although each factor recognizes a distinct ribosomal state, each must bind in such a way that the SRL interacts with its catalytic histidine. Therefore, GTPase activation by the SRL is the universal mechanism for triggering GTP hydrolysis on the ribosome across all kingdoms of life.

References and Notes

1. T. Margus, M. Remm, T. Tenson, *BMC Genomics* **8**, 15 (2007).
2. T. E. Dever, M. J. Glynias, G. C. Merrick, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1814 (1987).
3. D. Moazed, J. M. Robertson, H. F. Noller, *Nature* **334**, 362 (1988).
4. L. Holmberg, O. Nygård, *Biochemistry* **33**, 15159 (1994).
5. J. F. Eccleston, M. R. Webb, *J. Biol. Chem.* **257**, 5046 (1982).

6. H. Berchtold *et al.*, *Nature* **365**, 126 (1993).
 7. M. Kjeldgaard, P. Nissen, S. Thirup, J. Nyborg, *Structure* **1**, 35 (1993).
 8. E. Jacquet, A. Parmeggiani, *EMBO J.* **7**, 2861 (1988).
 9. T. Pape, W. Wintermeyer, M. V. Rodnina, *EMBO J.* **17**, 7490 (1998).
 10. J. R. Mesters, A. P. Potapov, J. M. de Graaf, B. Kraal, *J. Mol. Biol.* **242**, 644 (1994).
 11. T. P. Hausner, J. Atmadja, K. H. Nierhaus, *Biochimie* **69**, 911 (1987).
 12. H. R. Bourne, D. A. Sanders, F. McCormick, *Nature* **349**, 117 (1991).
 13. D. Mohr, W. Wintermeyer, M. V. Rodnina, *Biochemistry* **41**, 12520 (2002).
 14. L. Vogeley, G. J. Palm, J. R. Mesters, R. Hilgenfeld, *J. Biol. Chem.* **276**, 17149 (2001).
 15. T. M. Schmeing *et al.*, *Science* **326**, 688 (2009).
 16. J. Sengupta *et al.*, *J. Mol. Biol.* **382**, 179 (2008).
 17. E. Villa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 1063 (2009).
 18. J. C. Schuette *et al.*, *EMBO J.* **28**, 755 (2009).
 19. P. Nissen *et al.*, *Science* **270**, 1464 (1995).
 20. M. Valle *et al.*, *EMBO J.* **21**, 3557 (2002).
 21. D. J. Taylor *et al.*, *EMBO J.* **26**, 2421 (2007).
 22. C. Knudsen, H. J. Wieden, M. V. Rodnina, *J. Biol. Chem.* **276**, 22183 (2001).
 23. D. M. Blow, J. J. Birktoft, B. S. Hartley, *Nature* **221**, 337 (1969).
 24. M. Selmer *et al.*, *Science* **313**, 1935 (2006).
 25. N. Bilgin, M. Ehrenberg, *J. Mol. Biol.* **235**, 813 (1994).
 26. F. Rambelli *et al.*, *Biochem. J.* **259**, 307 (1989).
 27. J. M. Ogle *et al.*, *Science* **292**, 897 (2001).
 28. R. Mittal, M. R. Ahmadian, R. S. Goody, A. Wittinghofer, *Science* **273**, 115 (1996).
 29. K. Rittinger, P. A. Walker, J. F. Eccleston, S. J. Smerdon, S. J. Gamblin, *Nature* **389**, 758 (1997).
 30. J. J. Tesmer, D. M. Berman, A. G. Gilman, S. R. Sprang, *Cell* **89**, 251 (1997).
 31. M. Diaconu *et al.*, *Cell* **121**, 991 (2005).
 32. H. J. Wieden, W. Wintermeyer, M. V. Rodnina, *J. Mol. Evol.* **52**, 129 (2001).
 33. M. Remacha *et al.*, *Mol. Cell. Biol.* **15**, 4754 (1995).
 34. M. V. Rodnina, R. Fricke, W. Wintermeyer, *Biochemistry* **33**, 12267 (1994).
35. We thank R. Green for reagents and D. de Sanctis at the European Synchrotron Radiation Facility for facilitating data collection. This work was supported by the Medical Research Council UK, the Wellcome Trust, the Agouron Institute, and the Louis-Jeantet Foundation. R.M.V. is supported by a Gates-Cambridge scholarship, and T.M.S. by the Human Frontier Science Program and Emmanuel College. V.R. is on the Scientific Advisory Board and holds stock options in Rib-X pharmaceuticals. Transfer of *Thermus thermophilus* ribosome strain T.th. HB8-MRC-MSAW1 requires a Materials Transfer Agreement with the MRC. Coordinates and structure factors have been deposited at the Protein Data Bank with accession codes 2xqd and 2xqe.

Supporting Online Material

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Evolution of Yeast Noncoding RNAs Reveals an Alternative Mechanism for Widespread Intron Loss

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The evolutionary forces responsible for intron loss are unresolved. Whereas research has focused on protein-coding genes, here we analyze noncoding small nucleolar RNA (snoRNA) genes in which introns, rather than exons, are typically the functional elements. Within the yeast lineage exemplified by the human pathogen *Candida albicans*, we find—through deep RNA sequencing and genome-wide annotation of splice junctions—extreme compaction and loss of associated exons, but retention of snoRNAs within introns. In the *Saccharomyces* yeast lineage, however, we find it is the introns that have been lost through widespread degeneration of splicing signals. This intron loss, perhaps facilitated by innovations in snoRNA processing, is distinct from that observed in protein-coding genes with respect to both mechanism and evolutionary timing.

In eukaryotes, protein-coding genes are frequently interrupted by introns, which must be precisely removed from RNA transcripts by

the nuclear spliceosome (1). Over evolutionary time scales, the presence of introns is dynamic, with intron gain and loss rates varying substan-

tially across eukaryotic lineages (2–4). The mechanisms of intron gain and loss speak to questions about both the origins of introns and the markedly different intron-exon patterns observed across eukaryotes (5)—for example, whether spliceosomal introns arose within eukaryotes (“introns late”), within an ancestor of both prokaryotes and eukaryotes (“introns early”), or even before the emergence of protein-coding genes (“introns first”) (6). The last two hypotheses depend on the feasibility of comprehensive intron loss within both the prokaryotic and archaeal lineages, whose modern representatives lack spliceosomal introns. Within eukaryotes, the hemiascomycetous yeasts show substantial intron loss, with modern species like *Saccharomyces cerevisiae* and *Candida albicans* devoid of introns in >90% of their genes (7). A postulated mechanism for this loss is reverse transcription of spliced RNA, followed by homologous DNA recombination that replaces the intron-containing genomic sequence with the intronless copy (8). Previous studies of intron loss have focused on protein-coding genes, however, and are therefore likely to be biased toward mecha-

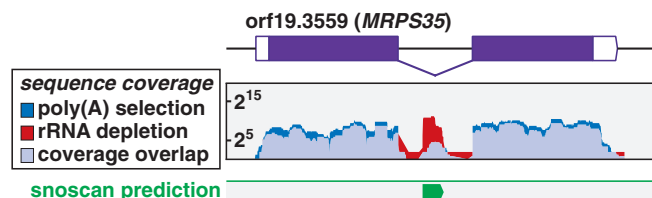
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Fig. 1. Sequence library comparisons reveal noncoding RNAs. RNA sequence data are shown for *MRPS35*, a representative protein-coding gene that hosts a snoRNA within its intron. Nonadenylated RNAs, such as mature snoRNAs, are enriched in the rRNA-depleted library relative to the poly(A)-selected library. Sequence depth is represented on a log₂ axis. (Bottom track) One of 1706 lower-confidence snoRNA predictions generated using the snoscan algorithm (22).



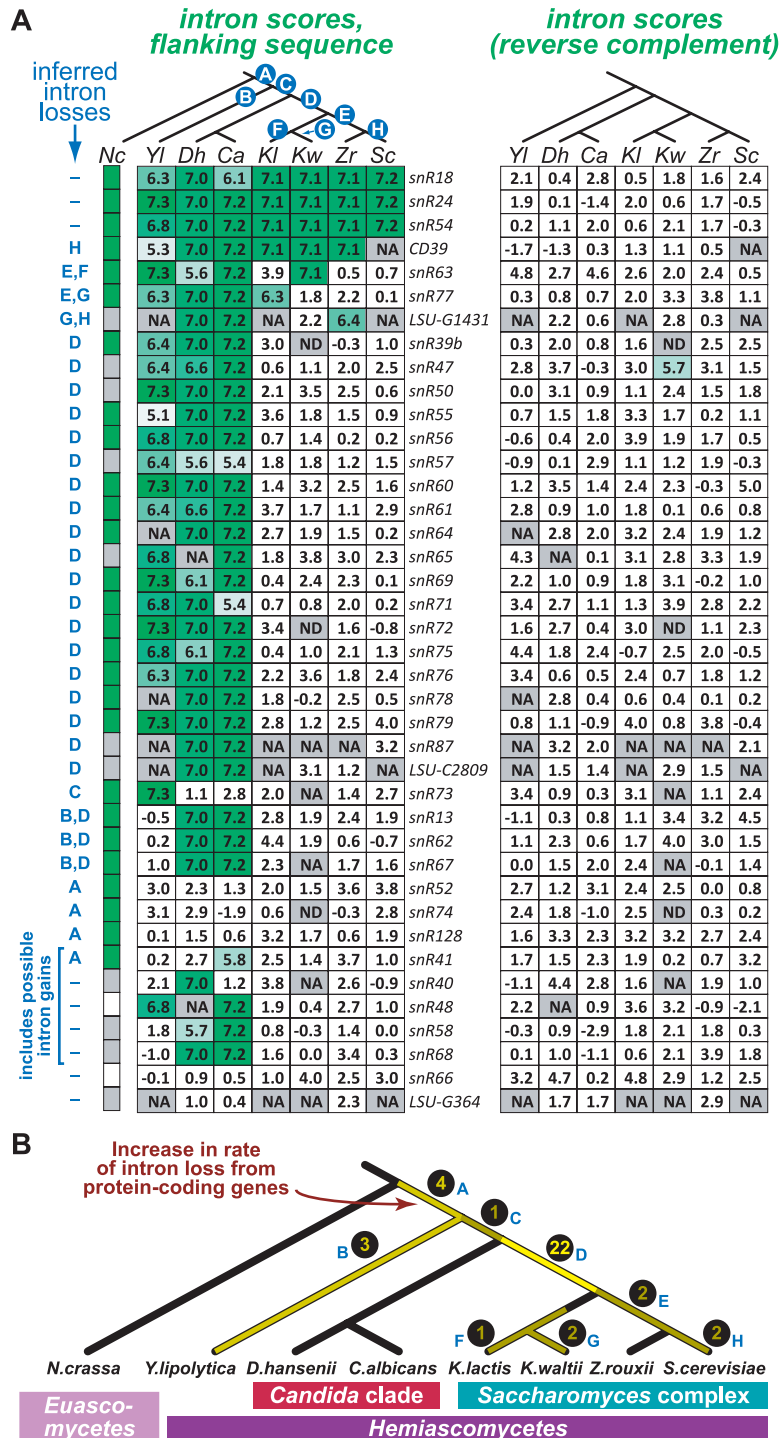


Fig. 2. snoRNA-associated introns were lost in the *Saccharomyces* lineage. **(A)** Intron prediction scores [combined 5' splice site and branch site species-specific PWM scores (15)] for 40 C/D box snoRNA flanking sequences (± 200 nt) in seven representative hemiascomycetes. Each snoRNA (horizontal row) is labeled according to the nomenclature of *S. cerevisiae* (where applicable) or *N. crassa* (CD39) or by the predicted *C. albicans* rRNA modification site. Intron scores greater than 5.0 (false-positive rate $<0.4\%$) are shaded green. Inferred intron loss events for each snoRNA, based on parsimony, are indicated on the left and correspond to labeled branches (not drawn to scale) in the phylogeny shown above and in **(B)**. snoRNAs that were not identified or that lack sufficient flanking sequence to score are gray and labeled NA or ND, respectively. Locations of *N. crassa* orthologs (green for intronic, white for exonic) are derived from (12). Intron scores for reverse-complements of flanking sequences (right) are provided as a negative control. **(B)** Phylogenetic pattern of snoRNA-associated intron loss. The number of loss events assigned to each branch is indicated in yellow. Species: *N. crassa*, *Y. lipolytica*, *Debaryomyces hansenii*, *C. albicans*, *Kluyveromyces lactis*, *Kluyveromyces waltii*, *Zygosaccharomyces rouxii*, and *S. cerevisiae*.

nisms that lead to precise intron removal. Here, we examine instead the evolution of splicing patterns in yeast noncoding genes.

We performed massively parallel ligation-based sequencing of RNA libraries (RNA-seq) and mapped the resulting ~ 160 million 50-nucleotide (nt) strand-specific sequence reads to the *C. albicans* genome (9). Our data confirm 89% of 421 previously annotated spliceosomal introns (7, 10) (table S1), while correcting or rejecting seven of these annotations (table S2). We also find 68 previously unannotated splice junctions, identifying 15 new introns in protein-coding genes (table S3), 30 examples of alternative splicing [table S4 and supporting online (SOM) text], and 23 new introns in previously unannotated transcripts that lack substantial protein-coding potential (table S5). Analysis of 11 of these spliced, noncoding RNAs revealed that their exons have no apparent function, but that their introns contain C/D box snoRNAs—noncoding RNAs that target modifications to ribosomal RNA (rRNA) (11). In the nonhemiascomycetous fungus *Neurospora crassa*, snoRNAs are also generally processed from the introns of non-protein-coding precursors (12). This is different, however, from the more closely related hemiascomycete *S. cerevisiae*, where nearly all snoRNAs arise from unspliced primary transcripts and, therefore, require a splicing-independent processing pathway (13). This difference between *C. albicans* and *S. cerevisiae* suggests that the transition of snoRNAs from intron sequences to unspliced, dedicated transcripts occurred within the *Saccharomyces* lineage, well after the onset of rapid intron loss from protein-coding genes in the hemiascomycete ancestor.

To trace the evolution of snoRNAs throughout the hemiascomycetes, we first generated 40 high-confidence C/D box snoRNA predictions for *C. albicans* (e.g., Fig. 1). Among these are the 11 identified in our intron analysis (above), as well as the previously identified *snR52* (14). We searched for splicing signals in sequences adjacent to snoRNAs, and predict that 33 of the 40 identified *C. albicans* snoRNAs are intronic. This confirms the difference between *C. albicans* and *S. cerevisiae*, as C/D box snoRNAs in the latter species are rarely intronic [6 out of 47 (13)].

We next identified orthologous snoRNAs from other sequenced yeasts (Fig. 2), beginning with computational predictions (or, for *S. cerevisiae*, existing annotations), identifying candidate orthologs of our *C. albicans* set based on their predicted rRNA target sites, and finally confirming and refining our predictions by searching for limited primary sequence identity among predicted snoRNAs (15). This final refinement identified snoRNAs whose rRNA target sites had changed between species, demonstrating evolutionary plasticity in yeast rRNA methylation sites (fig. S1). We predict that 105 of the 255 analyzed snoRNAs are located within introns (Fig. 2A). Individual species vary considerably: Those in the *Saccharomyces* complex have few intronic snoRNAs (three to five), whereas others have substantially more (23 to 33). The most par-

simonious explanation for these data is massive loss of snoRNA-associated introns, most of which took place in the common ancestor of the *Saccharomyces* complex (Fig. 2B).

The intron loss mechanism proposed for protein-coding genes—retrotransposition of spliced mRNAs—cannot explain the pattern we observe, as it would eliminate the snoRNAs along with the introns. Rather, the introns appear to have been lost through degeneration of their splicing signals, effectively converting them into unspliced exon sequence. In *N. crassa* (12) and hemiascomycetes outside the *Saccharomyces* complex, snoRNAs in the *snR72-78* polycistronic cluster are mostly contained within individual introns (Fig. 3A). In *S. cerevisiae*, the genes are arranged identically, but are cotranscribed as a single unspliced precursor, then processed into individual snoRNAs by the RNase III enzyme Rnt1 (16). The conservation of genomic synteny among these species strongly suggests intron loss through splice site degeneration (“de-intronization”) with *Yarrowia*

lipolytica and the *Candida* clade representing an intermediate state of partial intron loss and *S. cerevisiae* representing complete intron loss. The *snR57* cluster similarly supports this idea (below), as do the gene structures of *snR47* and *snR79* (SOM text).

Intron loss through splice-site degeneration would not be expected to occur within protein-coding genes, as this would disrupt the encoded protein. Consistent with this prediction, of the five snoRNAs located in introns within protein-coding regions in *C. albicans*, four remain associated with introns in nearly all the *Saccharomyces* complex species [*snR18*, *snR24*, *snR54*, and *CD39*; (Fig. 2A)]. The fifth, *snR39b*, was likely displaced from its associated coding sequence through a genomic duplication event (fig. S2B). The snoRNA *CD39* is located within the ribosomal protein gene *MRPS35* intron in all but one of the species we analyzed (Fig. 2A). The exception is *S. cerevisiae*, where *MRPS35* has lost its intron, presumably through retrotransposition

rather than deintronization. This appears to have eliminated *CD39* from the genome as well: The predicted rRNA methylation site for this snoRNA is unmodified in *S. cerevisiae* (13).

In *C. albicans*, one unusual case of splicing may reflect the particular processing requirements imposed by intron-hosted snoRNAs. The snoRNAs *snR57*, *snR55*, and *snR61* are processed from three introns of a single precursor (Fig. 3B). We find that two of the introns (introns 2 and 3) lie entirely within the sequence of an enveloping intron (intron 1). The splicing signals for intron 1 are not present in the primary transcript, but are created upon splicing of introns 2 and 3. Thus, splicing of intron 1 can occur only after introns 2 and 3 have been removed. (See SOM text for similar scenarios in protein-coding genes of other eukaryotes.) Analysis of both the *snR57/55/61* and the *snR72-78* clusters in other species indicates a significant reduction in the sizes of internal exons within the hemiascomycetes, driven perhaps by the same pressures that streamlined their genomes (17) and leading ultimately to nested splicing within the *Candida* clade (fig. S3). In animals and fungi, intron-hosted C/D box snoRNAs obey a strict “one-snoRNA-per-intron” rule, a requirement imposed by their exonucleolytic maturation pathways (18). Nested splicing of snoRNA host transcripts fulfills this requirement by allowing sequential removal of individual introns despite the absence of intervening exons.

Selective pressures are proposed to have driven intron loss from hemiascomycete protein-coding genes (19), and these same pressures may have driven the loss we observe here. The dependence of snoRNAs on splicing for their proper maturation, however, would have imposed a constraint against loss of their associated introns (18). This constraint may have been overcome by the innovation of an alternative snoRNA processing pathway in the *Saccharomyces* lineage, where exonic C/D box snoRNAs first undergo endonucleolytic cleavage by the RNase III enzyme Rnt1 (20). Nonetheless, some capacity to process exonic snoRNAs must be more ancient, because other hemiascomycetes (Fig. 2) and more distantly related fungi (12) do have some exon-hosted snoRNAs. It is unknown, however, whether processing of such snoRNAs involves Rnt1 (SOM text).

Studies of protein-coding genes have revealed a dramatic increase in the rate of intron loss in the hemiascomycete ancestor (21). By focusing instead on noncoding RNAs, we describe unexpected patterns of both exon and intron loss. A drastic reduction in the sizes of internal exons has ultimately led to their complete loss in *Candida* species, resulting in a complex form of splicing that maintains the independent removal of now overlapping introns. The *Saccharomyces* complex, however, has experienced a second wave of intron loss, perhaps facilitated by innovations in snoRNA processing and mediated by a mechanism—splice site degeneration—distinct from that which has acted on protein-coding genes.

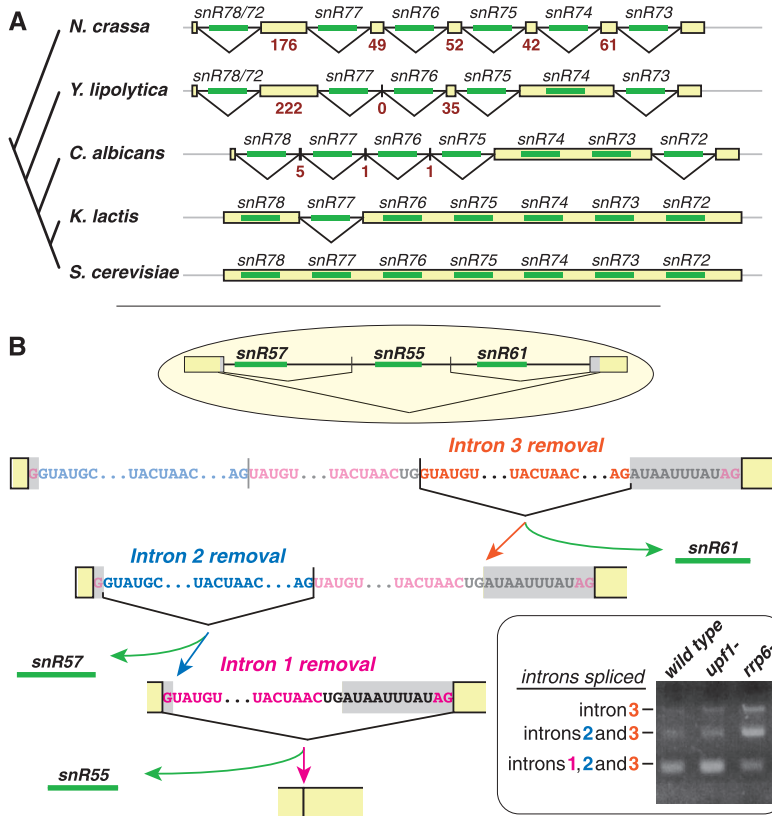


Fig. 3. Polycistronic snoRNA precursors exhibit unusual splicing patterns. (A) Splicing of the *snR72-78* cluster in various fungal species (phylogenetic relations on left). snoRNAs are shown in green, introns as lines, and exons as yellow boxes, with internal exons labeled by size. Cotranscription of entire clusters has been demonstrated only for *S. cerevisiae* (16) and *N. crassa* (12). (B) Nested splicing of the *snR57/55/61* snoRNA cluster in *C. albicans*. The 5' splice site, branch site, and 3' splice site sequences are shown for introns 1 (pink), 2 (blue), and 3 (orange). Gray "exons" represent sequences joined with intron 1, by splicing of introns 2 and 3, to create de novo intron 1 splice sites. (Inset) Reverse transcription polymerase chain reaction products of the snoRNA host transcript from wild-type cells and those deficient for nonsense-mediated mRNA decay (*upf1-Δ*) or nuclear exonucleolytic decay (*rrp6-Δ*). We infer the order of splicing events from the observable products: Intron 3 is nearly always removed, intron 2 is removed in a subset of transcripts (lower two bands), and intron 1 only when 2 and 3 have also been removed (lower band). Patterns in decay mutants are consistent with degradation of partially spliced host transcripts in the nucleus.

References and Notes

1. M. C. Wahl, C. L. Will, R. Lüthmann, *Cell* **136**, 701 (2009).
2. W. Li, A. E. Tucker, W. Sung, W. K. Thomas, M. Lynch, *Science* **326**, 1260 (2009).
3. L. Carmel, Y. I. Wolf, I. B. Rogozin, E. V. Koonin, *Genome Res.* **17**, 1034 (2007).
4. S. W. Roy, W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 5773 (2005).
5. S. W. Roy, W. Gilbert, *Nat. Rev. Genet.* **7**, 211 (2006).
6. D. Penny, M. P. Hoepfner, A. M. Poole, D. C. Jeffares, *J. Mol. Evol.* **69**, 527 (2009).
7. Q. M. Mitrovich, B. B. Tuch, C. Guthrie, A. D. Johnson, *Genome Res.* **17**, 492 (2007).
8. G. R. Fink, *Cell* **49**, 5 (1987).
9. B. B. Tuch *et al.*, *PLoS Genet.* **6**, e1001070 (2010).
10. B. R. Braun *et al.*, *PLoS Genet.* **1**, 36 (2005).
11. L. B. Weinstein, J. A. Steitz, *Curr. Opin. Cell Biol.* **11**, 378 (1999).
12. N. Liu *et al.*, *BMC Genomics* **10**, 515 (2009).
13. D. Piekna-Przybylska, W. A. Decatur, M. J. Fournier, *RNA* **13**, 305 (2007).
14. C. Marck *et al.*, *Nucleic Acids Res.* **34**, 1816 (2006).
15. Materials and methods are available as supporting material on Science Online.
16. L. H. Qu *et al.*, *Mol. Cell. Biol.* **19**, 1144 (1999).
17. B. Dujon *et al.*, *Nature* **430**, 35 (2004).
18. J. W. Brown, D. F. Marshall, M. Echeverria, *Trends Plant Sci.* **13**, 335 (2008).
19. M. Lynch, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 6118 (2002).
20. G. Chanfreau, P. Legrain, A. Jacquier, *J. Mol. Biol.* **284**, 975 (1998).
21. J. E. Stajich, F. S. Dietrich, S. W. Roy, *Genome Biol.* **8**, R223 (2007).
22. T. M. Lowe, S. R. Eddy, *Science* **283**, 1168 (1999).
23. We thank C. Barbacioru, O. Homann, S. Kuersten, S. Ledoux, I. Listerman, T. Lowe, C. Maeder, K. Pande, A. Price, S. Roy, J. Steitz, and members of the Guthrie and Johnson laboratories for helpful discussions and support; and M. Barker and C. Monighetti for providing sequence reads. This work was supported by NIH grants GM021119 (C.G.) and GM37049 (A.D.J.) and by Life Technologies Corp. C.G. is an American Cancer Society Research Professor of Molecular Genetics. Sequence data are available at the Gene Expression Omnibus (accession number GSE12191).

Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S4

Tables S1 to S6

References

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Fate Mapping Analysis Reveals That Adult Microglia Derive from Primitive Macrophages

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Microglia are the resident macrophages of the central nervous system and are associated with the pathogenesis of many neurodegenerative and brain inflammatory diseases; however, the origin of adult microglia remains controversial. We show that postnatal hematopoietic progenitors do not significantly contribute to microglia homeostasis in the adult brain. In contrast to many macrophage populations, we show that microglia develop in mice that lack colony stimulating factor-1 (CSF-1) but are absent in CSF-1 receptor-deficient mice. In vivo lineage tracing studies established that adult microglia derive from primitive myeloid progenitors that arise before embryonic day 8. These results identify microglia as an ontogenically distinct population in the mononuclear phagocyte system and have implications for the use of embryonically derived microglial progenitors for the treatment of various brain disorders.

Although microglial ontogeny is an extensive area of research, much controversy remains regarding the nature of microglial progenitors (1, 2). The most consensual hypothesis to date is that embryonic and perinatal

hematopoietic waves of microglial recruitment and differentiation occur in the central nervous system (CNS) (1, 2). However, the exact contribution of embryonic and postnatal hematopoietic progenitors to the adult microglial pool in the steady state remains unclear. We examined the contribution of primitive and definitive hematopoiesis to the adult microglial population that populates the CNS during normal development. Our results provide direct evidence that adult microglia derive from primitive myeloid progenitors that arise before embryonic day 8 (age E8.0) and for the predominant contribution of primitive myeloid progenitors to an adult hematopoietic compartment.

To address the contribution of perinatal circulating hematopoietic precursors to microglial homeostasis, we reconstituted sublethally irradiated C57BL/6 CD45.2⁺ newborns with hematopoietic cells isolated from CD45.1⁺ congenic mice (3). Although more than 30% circulating leukocytes and tissue macrophages were of donor origin 3 months after transplant (fig. S1A), 95% of adult microglia remained of host origin at

this time point (fig. S1, A and B). These results suggest that, in contrast to previous reports (4, 5), perinatal circulating hematopoietic precursors, including monocytes, do not substantially contribute to adult microglial homeostasis. With use of adult congenic bone marrow chimera models, evidence in favor of (6–8) and against (9, 10) the contribution of circulating hematopoietic cells to microglial homeostasis has been proposed. We found consistently that 10 to 20% of microglia in the brain parenchyma are of donor origin at 10, 15, and 21 months after transplant (fig. S1C). Parabiotic mice, which share the same blood circulation, provide a means to follow the turnover of adult circulating hematopoietic precursors without the need for exposure to radiation injuries. Although the mixing of the myeloid lineage is less efficient than the mixing of the lymphoid lineage (11), an average of 30% of monocytes and tissue macrophages were donor-derived at 1 month and 12 months after parabiosis (fig. S1D) (12). In contrast, less than 5% of microglia were donor-derived at these time points (fig. S1D), in agreement with a previous report on 5-month-old parabionts (9). Consistent with previous reports (9, 10, 13), these results suggest that the recruitment of bone marrow-derived cells to the brain of chimeric animals is dependent on radiation-induced brain injuries that followed the transplantation regimen. These results also suggest that postnatal microglia are maintained independently of circulating monocytes throughout life and are maintained by local radio-resistant precursors that colonize the brain before birth.

Next, we examined the origin of microglia during development. In mouse embryos, the first wave of hematopoietic progenitors appears in the extra-embryonic yolk sac and leads to the production of primitive hematopoiesis, which takes place between E7.0 and E9.0 (14, 15). An independent wave of hematopoiesis termed “definitive hematopoiesis” is initiated within the embryo proper in the aorta, gonads, and mesonephros (AGM) region (14, 15). Around E10.5, hematopoietic progenitors start to colonize the fetal liver, which serves as a major hematopoietic organ after E11.5, whereas later during develop-

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ment hematopoiesis takes place in the spleen and bone marrow (15). Tissue macrophages in the adult are thought to derive from bone marrow monocytes, and the contribution of primitive hematopoiesis to adult tissue macrophages remains unclear (16). To determine the developmental stage at which the seeding of myeloid cells occurs in the brain, we used *Cx3cr1^{gfp/+}* knockin mice (17) because the fractalkine receptor (CX3CR1) is a marker of early myeloid progenitors (18) and microglia (19). Consistent with previous results (20), myeloid cells expressing the hematopoietic marker CD45 and the adult macrophage/microglia markers CD11b, F4/80, and CX3CR1 were detectable in the

developing brain starting from E9.5 (Fig. 1, A and B and fig S2). At E10.5, microglia cells were present in both the cephalic mesenchyme and the neuroepithelium, although at a lower density in the latter (Fig. 1A, fig. S2, and movies S1 and S2). The phenotype of microglial cells resembled that of yolk sac macrophages throughout embryonic development (Fig. 1, B and C, and fig. S3). Analysis of the DNA content of microglia and in vivo live imaging indicated that they were highly proliferative throughout embryonic life (fig. S4 and movie S3).

The differentiation of most macrophage populations in adult mice is controlled by colony stimulating factor-1 (CSF-1) and its receptor

(CSF-1R) (21), but the role of the CSF-1 and CSF-1R in the development of yolk sac primitive macrophages and microglia is unknown. We found that CSF-1R was expressed in similar amounts on yolk sac macrophages and microglia at E9.5 (Fig. 2A). CSF-1R expression on microglia was maintained throughout development (Fig. 2A and fig. S5). Consistent with a requirement for CSF-1R expression, absence of CSF-1 greatly reduced the development of microglia (Fig. 2B and fig. S6A) and yolk sac macrophages (Fig. 2C and fig. S6B), whereas circulating monocytes were present in these mice (fig. S6, C and D). Furthermore, microglia remained largely absent

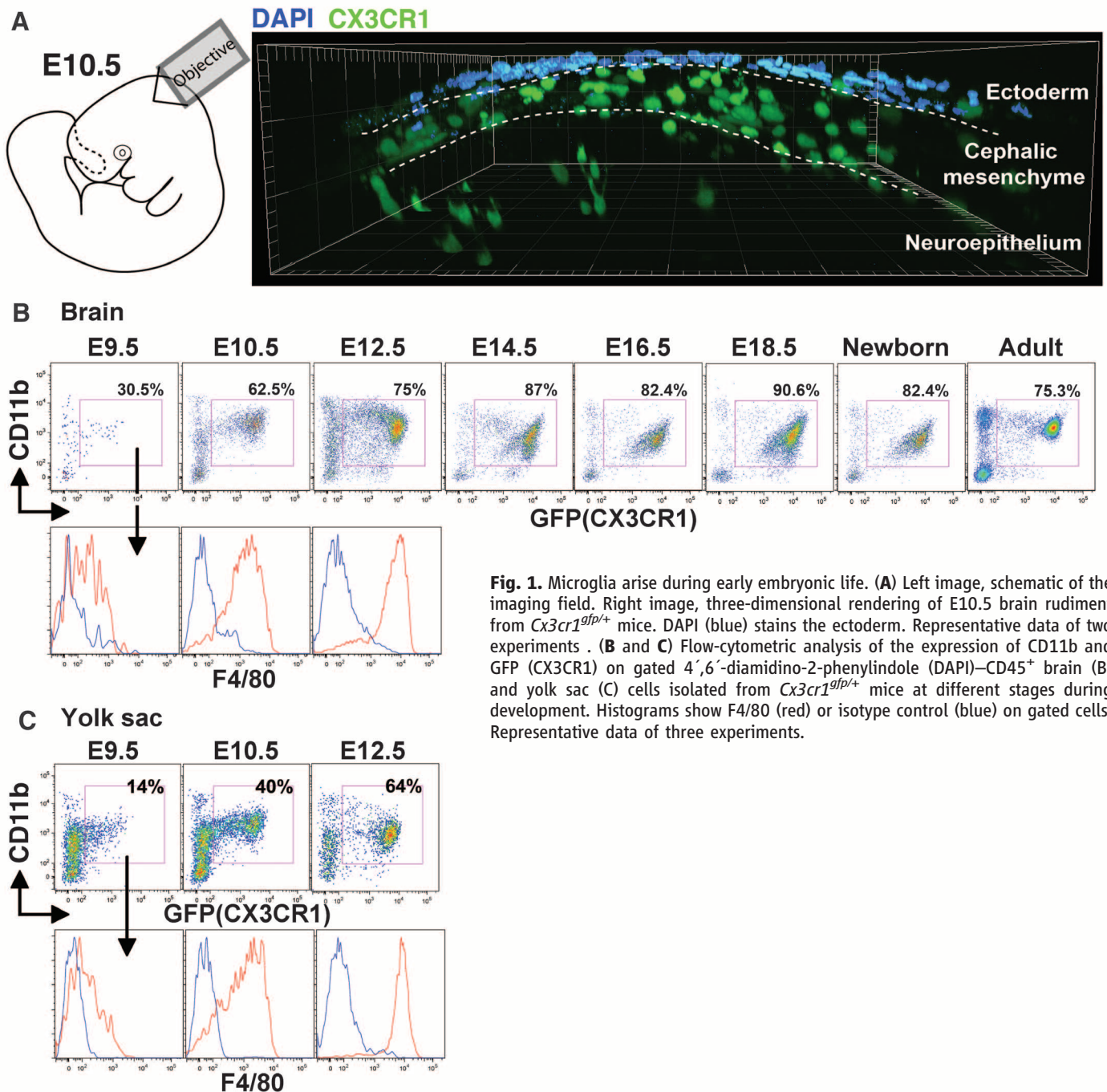


Fig. 1. Microglia arise during early embryonic life. (A) Left image, schematic of the imaging field. Right image, three-dimensional rendering of E10.5 brain rudiment from *Cx3cr1^{gfp/+}* mice. DAPI (blue) stains the ectoderm. Representative data of two experiments. (B and C) Flow-cytometric analysis of the expression of CD11b and GFP (CX3CR1) on gated 4',6'-diamidino-2-phenylindole (DAPI)-CD45⁺ brain (B) and yolk sac (C) cells isolated from *Cx3cr1^{gfp/+}* mice at different stages during development. Histograms show F4/80 (red) or isotype control (blue) on gated cells. Representative data of three experiments.

throughout life in CSF-1R-deficient mice (Fig. 2B and fig. S6A). All together, these results suggest that the development of yolk sac macrophages and microglia, but not monocytes, is strongly dependent on CSF-1R. In contrast to many tissue

macrophages, adult microglia can still form, albeit at reduced levels in *Csf-1^{op/op}* mice, which carry a natural null mutation of the *Csf-1* gene (22), but are absent in mice that lack CSF-1R (Fig. 2D). A second CSF-1R ligand, interleukin 34 (IL-34), has

recently been identified in mice, humans (23), and birds (24). The expression of IL-34 mRNA in the brain is much higher than the expression of CSF-1 mRNA during early postnatal development and in the adult (25), consistent with an important role for

Fig. 2. Microglia and yolk sac macrophages are absent in *Csf-1^{-/-}* mice. (A) Flow-cytometric analysis of CSF-1R expression (red) on microglia and yolk sac macrophages (blue, isotype control). Representative data of three experiments. (B and C) Percentage of microglia (B) and yolk sac macrophages (C) in *Csf-1^{-/-}* (black squares) or control littermate (*Wt*) (white squares) FVB/NJ mice. Pooled data from three separate experiments. $**P < 0.001$; $***P < 0.0001$. (D) Coronal sections of 3-week-old *Wt*, *Csf-1^{op/op}*, and *Csf-1^{-/-}* brains of region boxed in the schematic stained for the microglial marker Iba1. DG indicates dentate gyrus; Cx, cerebral cortex; CA3, CA3 region of the hippocampus. Mean number of Iba1⁺ cells per field from three different brain regions is shown. Average of six fields (0.5 mm²) per region per genotype. Error bars represent mean $\pm$ SD of data from two pooled experiments. $*P < 0.05$; $****P < 0.00001$.

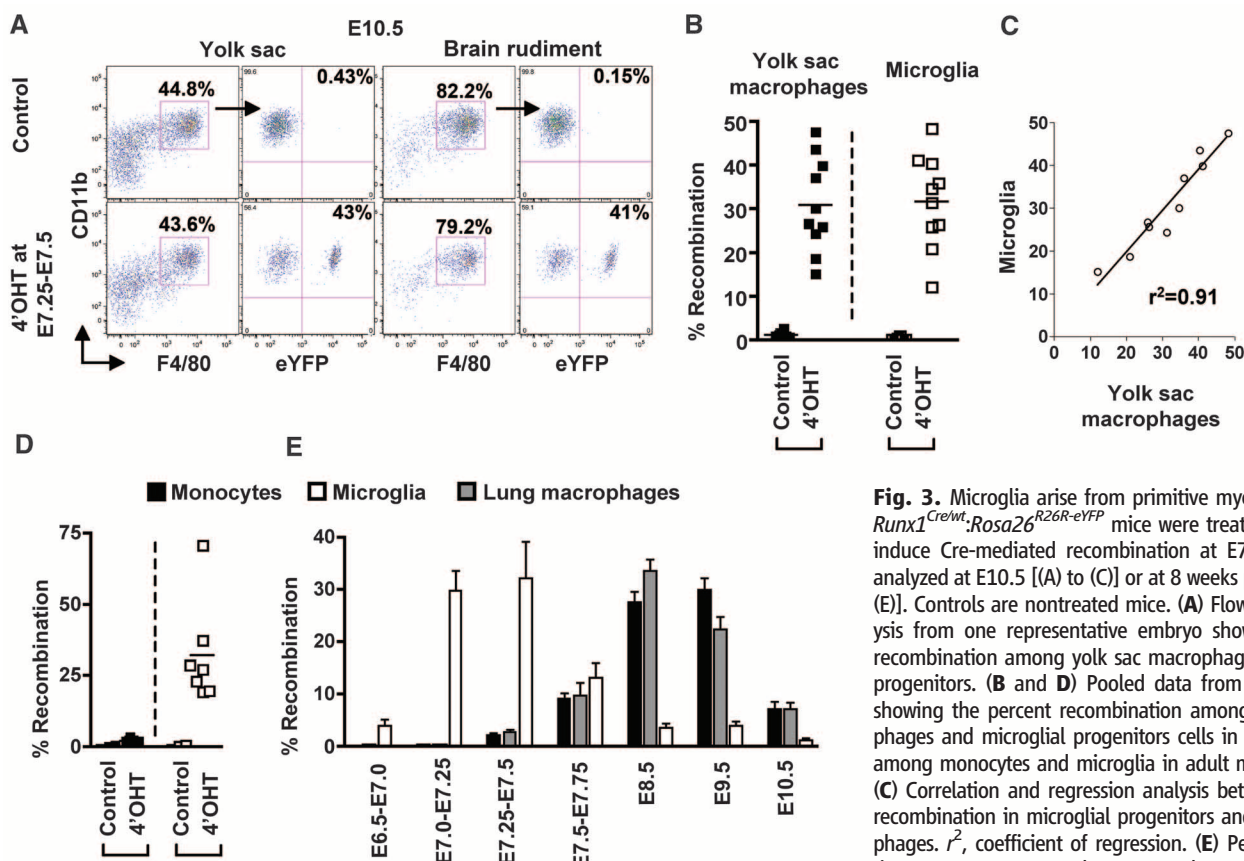
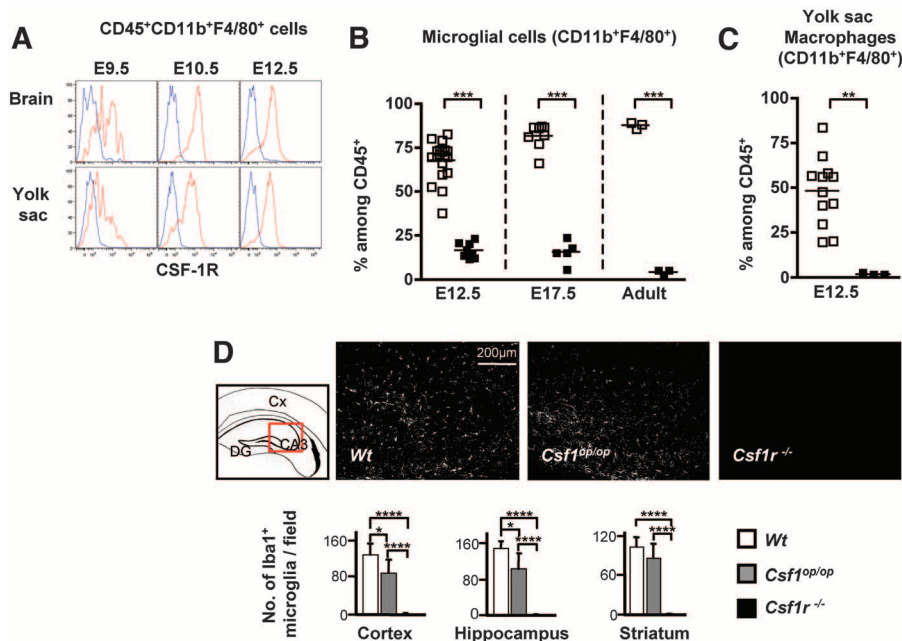
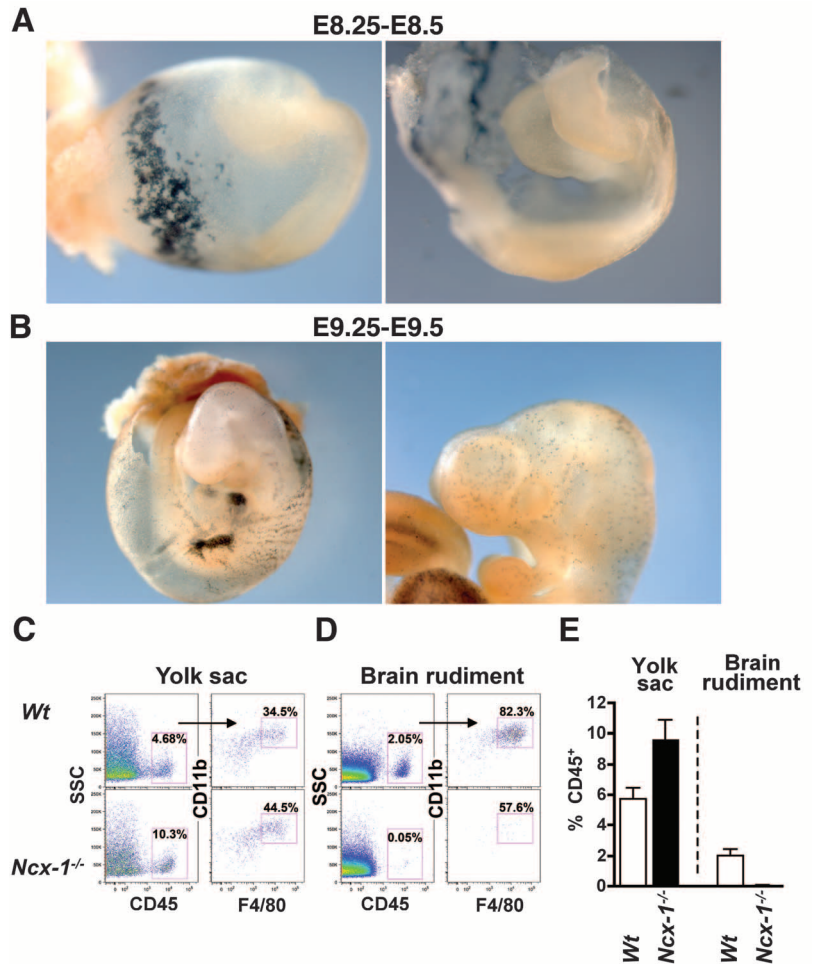


Fig. 3. Microglia arise from primitive myeloid progenitors. *Runx1^{Cre/wt}; Rosa26^{R26R-eYFP}* mice were treated with 4'OHT to induce Cre-mediated recombination at E7.25 to E7.5 and analyzed at E10.5 [(A) to (C)] or at 8 weeks postbirth [(D) and (E)]. Controls are nontreated mice. (A) Flow-cytometric analysis from one representative embryo showing the percent recombination among yolk sac macrophages and microglial progenitors. (B and D) Pooled data from two experiments showing the percent recombination among yolk sac macrophages and microglial progenitors cells in embryos (B), and among monocytes and microglia in adult mice ($n = 10$) (D). (C) Correlation and regression analysis between the percent recombination in microglial progenitors and yolk sac macrophages. r^2 , coefficient of regression. (E) Percent recombination among monocytes, lung macrophages, and microglia in

adult mice activated at different embryonic age. Error bars represent mean $\pm$ SEM of pooled data from two experiments ($n = 8$ to 16). Gating strategy for each leukocyte population is detailed in fig. S8.

Fig. 4. $Runx1^{+}$ yolk sac progenitors seed the brain between E8.5 and E9.5 through blood circulation. (A and B) $Runx1^{Cre/wt};Rosa^{26R26R-LacZ}$ embryos activated at E7.25 to E7.5 were isolated at E8.25 to E8.5 (A) or E9.25 to E9.5 (B) and processed for whole-mount LacZ staining as described in the materials and methods section. At E8.25 to E8.5, labeled cells are detected in the yolk sac but not in the brain rudiment or in the neural tube (A), whereas labeled cells infiltrate the brain rudiment of E9.25 to E9.5 embryos (B). (C to E) Yolk sac and brain rudiment tissues were isolated from E10.0 to E10.5 $Ncx1^{-/-}$ embryos or control littermates and processed for flow cytometry analysis as described in the materials and methods section. Dot plots show the presence of yolk sac macrophages in $Ncx1^{-/-}$ embryos and control littermates (C), whereas microglia were present in control but not in $Ncx1^{-/-}$ embryos (D). (E) The percentage $\pm$ SEM of hematopoietic cells (CD45 $^{+}$) in control littermates (white bars, $n = 4$) and $Ncx1^{-/-}$ embryos (black bars, $n = 3$).



IL-34 in the regulation of microglial homeostasis and with the increased severity of the microglial phenotype in $Csf1r^{-/-}$ compared with $Csf1r^{op/op}$ mice.

To examine the potential contribution of primitive myeloid precursors to adult microglia in vivo, we performed lineage tracing studies by using mice that express the tamoxifen-inducible $MER-Cre-MER$ recombinase gene under the control of one of the endogenous promoters of the runt-related transcription factor 1 ($Runx1$) locus. $Runx1$ expression is first seen at E6.5 and is strongly up-regulated around E7.5 in the proximal visceral yolk sac region, and, until E8.0, $Runx1^{+}$ cells are restricted to the extra-embryonic yolk sac and are absent from the embryo proper or allantois (26). We crossed the $Runx1-MER-Cre-MER$ mice ($Runx1^{Cre/wt}$) with the Cre-reporter mouse strain $Rosa^{26R26R-eYFP/R26R-eYFP}$ and induced recombination by a single injection of 4-hydroxytamoxifen (4'OHT) into pregnant females at different days of gestation. Active recombination in these knockin mice occurs in a small time window that does not exceed 24 hours postinjection (26) and leads to the irreversible expression of the enhanced yellow fluorescent protein (eYFP) reporter gene in $Runx1^{+}$ cells and their progeny. At E10.5, embryos activated at E7.25 to E7.5 exhibited a similar proportion of

labeled yolk sac macrophages and microglia [$31\% \pm 10.8$ (SE) for yolk sac macrophages and $32\% \pm 10.7$ for brain progenitors, $n = 10$] (Fig. 3, A and B). The proportion of brain-infiltrating hematopoietic cells and microglia was similar in control littermate and heterozygote $Runx1^{Cre/wt}$ embryos, suggesting that microglia homeostasis was not perturbed in $Runx1^{Cre/wt}$ embryos (fig. S7, A and B). Furthermore, the proportion of eYFP $^{+}$ microglia was similar in E10.5 and E13.5 embryos activated at E7.25 to E7.5 (fig. S7C), and within individual embryos the proportions of eYFP $^{+}$ yolk sac macrophages and eYFP $^{+}$ microglia were highly correlated (Fig. 3C). The partial labeling of $Runx1^{+}$ yolk sac cells is inherent to in vivo labeling techniques and likely results from the insufficient expression of $MER-Cre-MER$ and limited availability of the ligand in target cells (26). Thus, our model likely underestimates the contribution of $Runx1^{+}$ precursors to adult microglia homeostasis, although we cannot exclude the potential contribution of nonlabeled precursors to this process. Nevertheless, these results strongly suggest that yolk sac macrophages and microglia have the same origin and that the first wave of microglia is specified before the end of E8.0. In adult mice activated at E7.25 to E7.5 ($n = 10$ from 3 litters), $32.11\% \pm 18.0$ of microglia were also eYFP $^{+}$ (Fig. 3D and

fig. S8), a proportion similar to that of yolk sac macrophages and microglia found in E10.5 and E13.5 embryos activated at E7.25 to E7.5 (Fig. 3C and fig. S7). In contrast, less than 3% of blood circulating and tissue macrophages—including dermal and lung macrophages, as well as circulating T cells, B cells, and granulocytes—were eYFP $^{+}$ in these mice (Fig. 3E and figs. S8 and S9). We also examined the earliest stage at which $Runx1^{+}$ progenitors contributed to the adult microglial pool. In adult mice activated at E6.5 to E7.0, less than $3.77\% \pm 3.54$ microglia were eYFP $^{+}$, whereas $29.6\% \pm 10$ microglia were eYFP $^{+}$ in adult mice activated at E7.0 to E7.25 (Fig. 3E). In contrast $0.19\% \pm 0.26$ and $0.09\% \pm 0.05$ circulating leukocytes were eYFP $^{+}$ in adult mice activated at E6.5 to E7.0 and E7.0 to E7.25, respectively (fig. S9). These results establish that primitive myeloid progenitors that arise before E7.5 contribute significantly to adult microglial homeostasis in the healthy brain but have limited potential to give rise to adult blood leukocytes.

To determine at which stage $Runx1^{+}$ precursors or their progeny seed the brain during development, we injected $Runx1^{Cre/wt};Rosa^{26R26R-LacZ}$ pregnant females with a single dose of 4'OHT at E7.25 to E7.5 and traced the apparition of labeled cells into the brain rudiment at different time points after injection. A large number of LacZ $^{+}$

cells populated the yolk sac of E8.5 embryos, whereas no labeled cells were detectable in the brain rudiment at this time point (Fig. 4A and fig. S10A). Brain-infiltrating cells appeared only when blood circulation developed, and a significant proportion of Lac-Z⁺ cells appeared associated with blood vessels and infiltrated the brain rudiment in E9.5 conceptus (Fig. 4B and fig. S10B). These results are consistent with prior findings showing that CSF-1R⁺ cells first accumulate in the yolk sac around E8.0 and infiltrate the embryo proper when blood vessels develop around E9.0 (27). To address whether the development of functional blood vessels was required for the recruitment of myeloid precursors into the brain rudiment, we used *Ncx-I*^{-/-} animals that lack a heartbeat and functional blood circulation because of a defect in sodium calcium exchanger 1 (28). We found that E9.5 to E10.5 *Ncx-I*^{-/-} embryos have yolk sac macrophages levels comparable or higher than control littermates (Fig. 4, C and E). In contrast, *Ncx-I*^{-/-} embryos have no detectable microglia in the brain, whereas *Ncx-I*^{+/+} control littermates already have a substantial number of microglia in the brain at this time-point (Fig. 4, D and E). Altogether, these results suggest that Runx1⁺ progenitors migrate from the yolk sac into the brain through blood vessels between E8.5 and E9.5.

To examine the contribution of definitive hematopoiesis to microglial homeostasis, we injected 4'OHT at E8.5, E9.5, and E10.5. The proportion of eYFP⁺ leukocytes known to derive from definitive hematopoiesis was much higher in mice activated at E8.5 and E9.5 compared with mice activated at E7.25 to E7.5 (up to 40% versus less than 3%, *n* = 10) (Fig. 3E and fig. S9). In contrast, few eYFP⁺ microglia were detected in the brains of adult mice activated after E8.5 and onward (Fig. 3E). The sharp descending contribution levels between E7.5 and E8.5 argue against the contribution of post-E7.5 Runx1⁺ anatomic locations to the labeling of the adult microglia lineage. Altogether, these data suggest minimal, if any, contribution of definitive hematopoiesis to the development of adult microglia.

Our results provide evidence that primitive myeloid precursors give rise to microglia residing in the adult CNS in the steady state. Primitive macrophages differentiate in the yolk sac of mammals, birds, and zebrafish before the onset of blood circulation (14). Studies in zebrafish revealed that yolk sac-derived macrophages spread in the cephalic mesenchyme before invading the brain through the pial surfaces and the fourth ventricle (29–31), findings consistent with the observation that microglia in humans are formed in the pia mater (32). The conservation of primitive macrophages throughout evolution implies that they serve an important role in the early embryo (14), most likely related to the clearance of apoptotic bodies and the normal remodeling of brain tissues. In contrast to most adult tissue macrophages, microglia are maintained throughout life independently of any blood input and can

resist high-dose γ -ray irradiation. Whether these primitive macrophages are uniquely suited to reducing the risk of inflammation-induced injuries and maintaining the CNS integrity throughout adult life will be important to determine.

The results of this study should help unravel the regulatory program that controls microglia differentiation and function in vivo and identify new means to manipulate microglia for the treatment of neural diseases.

References and Notes

- W. Y. Chan, S. Kohsaka, P. Rezaie, *Brain Res. Brain Res. Rev.* **53**, 344 (2007).
- R. M. Ransohoff, V. H. Perry, *Annu. Rev. Immunol.* **27**, 119 (2009).
- Materials and methods are available as supporting material on Science Online.
- E. A. Ling, *J. Anat.* **128**, 847 (1979).
- S. K. Leong, E. A. Ling, *Glia* **6**, 39 (1992).
- L. J. Lawson, V. H. Perry, S. Gordon, *Neuroscience* **48**, 405 (1992).
- J. Priller et al., *Nat. Med.* **7**, 1356 (2001).
- A. R. Simard, D. Soulet, G. Gowing, J. P. Julien, S. Rivest, *Neuron* **49**, 489 (2006).
- B. Ajami, J. L. Bennett, C. Krieger, W. Tetzlaff, F. M. Rossi, *Nat. Neurosci.* **10**, 1538 (2007).
- A. Mildner et al., *Nat. Neurosci.* **10**, 1544 (2007).
- K. Liu et al., *Nat. Immunol.* **8**, 578 (2007).
- M. Bogunovic et al., *J. Exp. Med.* **203**, 2627 (2006).
- L. Vallières, P. E. Sawchenko, *J. Neurosci.* **23**, 5197 (2003).
- A. M. Lichanska, D. A. Hume, *Exp. Hematol.* **28**, 601 (2000).
- S. H. Orkin, L. I. Zon, *Cell* **132**, 631 (2008).
- R. van Furth, Z. A. Cohn, *J. Exp. Med.* **128**, 415 (1968).
- S. Jung et al., *Mol. Cell. Biol.* **20**, 4106 (2000).
- K. Liu et al., *Science* **324**, 392 (2009); 10.1126/science.1170540.
- J. K. Harrison et al., *Proc. Natl. Acad. Sci. U.S.A.* **95**, 10896 (1998).
- F. Alliot, I. Godin, B. Pessac, *Brain Res. Dev. Brain Res.* **117**, 145 (1999).

- V. Chitu, E. R. Stanley, *Curr. Opin. Immunol.* **18**, 39 (2006).
- G. Blevins, S. Fedoroff, *J. Neurosci. Res.* **40**, 535 (1995).
- H. Lin et al., *Science* **320**, 807 (2008).
- V. Garceau et al., *J. Leukoc. Biol.* **87**, 753 (2010).
- S. Wei et al., *J. Leukoc. Biol.* **88**, 495 (2010).
- I. M. Samokhvalov, N. I. Samokhvalova, S. Nishikawa, *Nature* **446**, 1056 (2007).
- D. A. Ovchinnikov et al., *J. Leukoc. Biol.* **83**, 430 (2008).
- S. V. Koushik et al., *FASEB J.* **15**, 1209 (2001).
- S. P. Sorokin, R. F. Hoyt Jr., D. G. Blunt, N. A. McNelly, *Anat. Rec.* **232**, 527 (1992).
- P. Herbomel, B. Thisse, C. Thisse, *Dev. Biol.* **238**, 274 (2001).
- P. Herbomel, B. Thisse, C. Thisse, *Development* **126**, 3735 (1999).
- M. A. Cuadros, J. Navascués, *Prog. Neurobiol.* **56**, 173 (1998).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1194637/DC1
Materials and Methods

Figs. S1 to S10

References

Movies S1 to S3

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Functional Compartmentalization and Viewpoint Generalization Within the Macaque Face-Processing System

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Primates can recognize faces across a range of viewing conditions. Representations of individual identity should thus exist that are invariant to accidental image transformations like view direction. We targeted the recently discovered face-processing network of the macaque monkey that consists of six interconnected face-selective regions and recorded from the two middle patches (ML, middle lateral, and MF, middle fundus) and two anterior patches (AL, anterior lateral, and AM, anterior medial). We found that the anatomical position of a face patch was associated with a unique functional identity: Face patches differed qualitatively in how they represented identity across head orientations. Neurons in ML and MF were view-specific; neurons in AL were tuned to identity mirror-symmetrically across views, thus achieving partial view invariance; and neurons in AM, the most anterior face patch, achieved almost full view invariance.

Primates can recognize faces accurately despite a plethora of transformations in size, position, makeup, illumination, and, perhaps the most drastic in terms of low-level feature characteristics, head orientation (1). A biolog-

ical substrate for primate face recognition is likely provided by face-selective cells (2–8) and by face-selective brain regions, which can be identified by functional magnetic resonance imaging (fMRI) experiments (9–12). In macaques,

fMRI reveals six discrete face-selective regions, consisting of one posterior face patch [posterior lateral (PL)], two middle face patches [middle

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lateral (ML) and middle fundus (MF)], and three anterior face patches [anterior fundus (AF), anterior lateral (AL), and anterior medial (AM)], spanning the entire extent of the temporal lobe (12). Why are there multiple face patches? Answering this question requires understanding the representation of faces in each patch. The six patches form strong, specific connections to each other (13). This suggests that the representations in each distinct patch are not inde-

pendent but constitute transformations of each other. In particular, electrical microstimulation in the middle face patches activates both AL and AM. Determining how ML, MF, AL, and AM represent faces was the goal of the current study.
We first used fMRI to localize face patches in two monkeys (M1 and M2). Both animals exhibited the typical arrangement of six face patches along the temporal lobe (Fig. 1A and

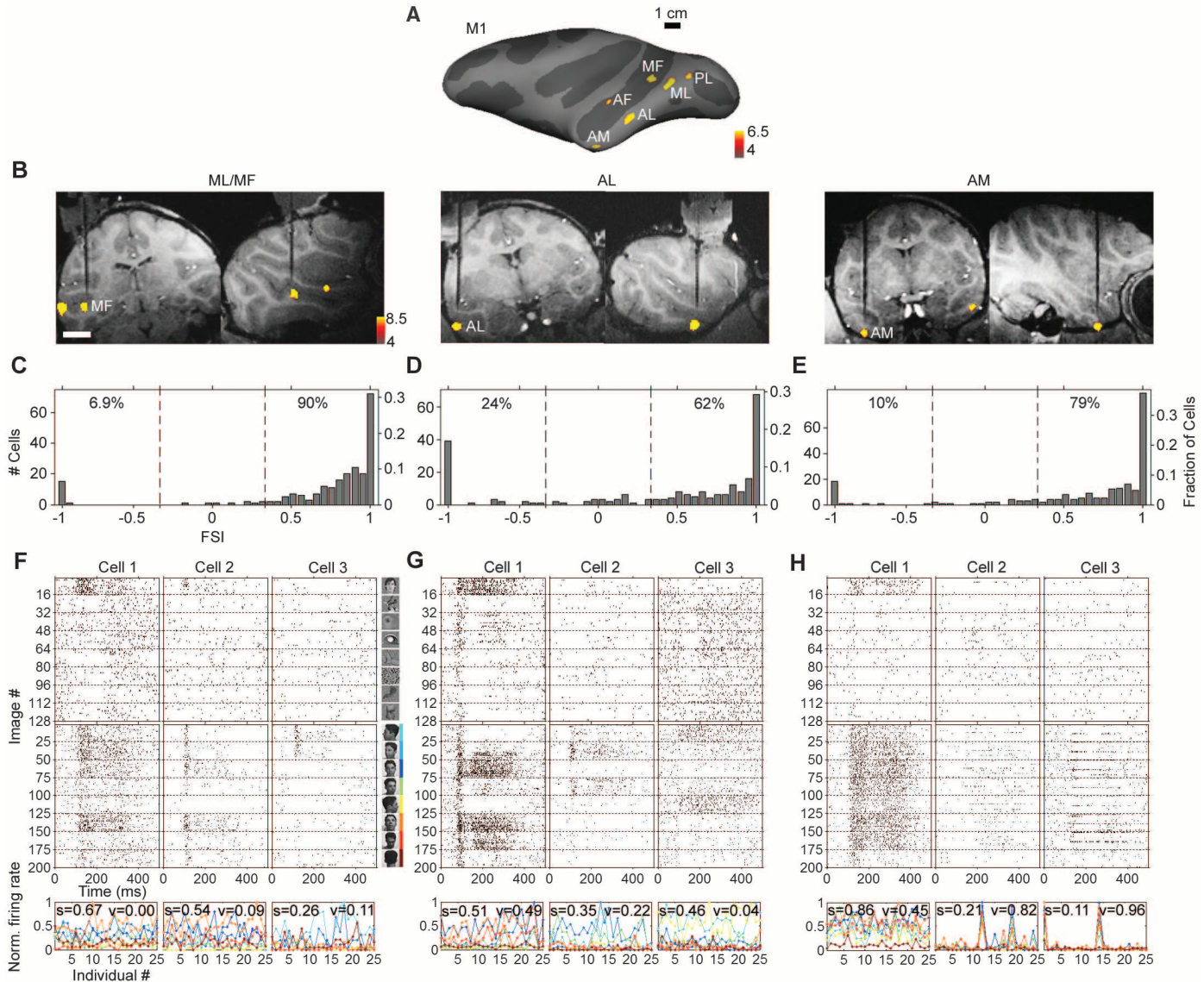


Fig. 1. Face selectivity in different parts of the macaque temporal lobe. (A) Inflated macaque left hemisphere (dark gray areas mark sulci, light gray–dark gray boundaries mark the middle of the bank within a sulcus) showing six regions in the temporal lobe of monkey M1 that responded significantly more to faces than to objects in fMRI experiments. Color scale indicates negative common logarithm of the *P* value. (B) Coronal (left) and sagittal (right) anatomical fMRI images showing the electrode descending into MF (located 3 mm anterior to the interaural line, AP (anterior-posterior) +3 mm), AL (at AP +12 mm), and AM (at AP +19 mm), respectively, in monkey M1. Coregistered face-selective functional activation is overlaid on the fMRI images. (C to E) Face selectivity of neural population responses in ML/MF, AL, and AM, respectively. Shown are distributions of face selectivity indices (FSIs) (see SOM) for visually responsive

cells in ML/MF, AL, and AM; dotted lines indicate FSI of ± 0.33 , corresponding to 1:2 and 2:1 response ratios to faces versus nonface objects. (F to H) Mean response time courses of three typical cells to the 128-image FOB set (top) and the 200-image FV set (middle) in ML/MF (F), AL (G), and AM (H), respectively. For clarity, responses are shown using a binary color scale. For the FV data, the first 25 rows are responses to 25 individuals looking to the left at full profile, the next 25 rows are responses to the same 25 individuals looking to the left at half profile, and so on; the eight different views of one example individual are shown on the right of (F). [Colored traces at the bottom of (F) to (H)]: Mean response levels to the 25 individuals at each head orientation, with the color corresponding to each view indicated in (F); *s* and *v* denote sparseness and view-invariant identity correlation coefficients, respectively (see SOM).

fig. S1A). We then targeted ML, MF, AL, and AM (Fig. 1B, fig. S1B, and table S1) for electrophysiological recordings. These face areas are defined solely by their anatomical locations. We found that response properties of cells within a given anatomically defined face patch, for example, AL, were highly similar across animals. Due to similarity of results across animals, we present combined results from the two animals; due to similarity of results from ML and MF, we group them together as ML/MF.

To compare the face selectivity of ML/MF, AL, and AM, we first recorded neural responses to the 128-image set used in the fMRI localizer experiments, consisting of 16 pictures each of eight

object categories (human faces, human bodies, fruits and vegetables, gadgets, human hands, scrambled patterns, monkey body parts, and monkey whole bodies), which we will refer to as the FOB (faces, objects, and bodies) stimulus set. We recorded from every cell encountered (table S1), and the analyses presented below include all cells for which we were able to obtain complete data from FOB as well as a second image set described below. In ML/MF, most cells responded more strongly to faces than to nonface objects: 97% of visually responsive cells were face selective (Fig. 1C and fig. S2A), with 90% selectively enhanced by faces (average face response at least twice as high as average nonface response) [see support-

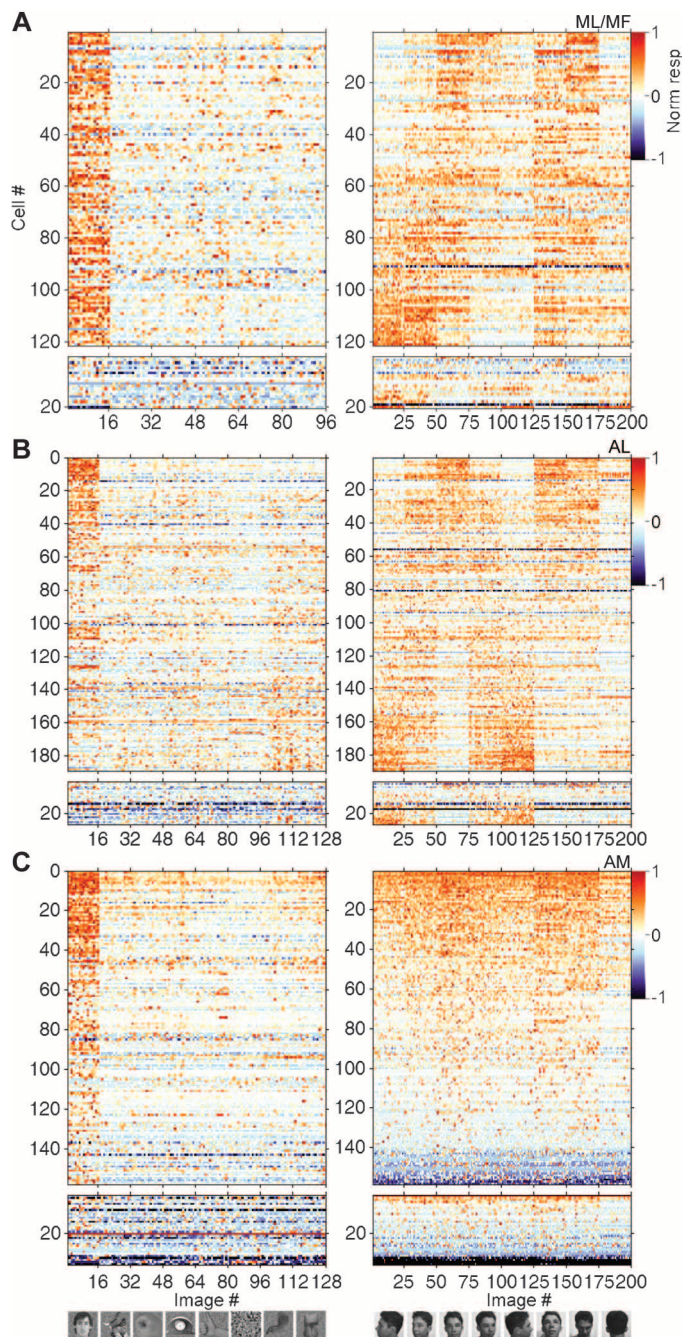
ing online material (SOM)] and 7% selectively suppressed by faces.

Both AL and AM contained large fractions of face-selective cells as well (86% in AL, 89% in AM) (Fig. 1, D and E). However, the selectivity patterns in AL and AM (fig. S2, B and C) differed from that in ML/MF: in AL, a much larger fraction of cells than in ML/MF was selectively suppressed by faces (24% versus 7%) or appeared unselective (14% versus 3%) (Fig. 1D). Similarly, in AM, relatively more cells were selectively face-suppressed (10%) or appeared non-selective (11%) (Fig. 1E). These results are puzzling because (i) anatomy suggests that AL and AM receive their major inputs from ML/MF (13, 14) and (ii) blood flow changes in AM and AL are as face selective as those in ML/MF (12). Therefore, one would expect AL and AM to inherit or even enhance the strong face selectivity of ML/MF.

Next, we probed cells with a second image set consisting of 200 pictures of 25 individuals each at eight different head orientations (left full profile, left half profile, straight, right half profile, right full profile, up, down, and back), a stimulus set that we will refer to as the FV (face views) set. Data obtained using the FV set from each of the three patches is presented side by side in Figs. 1, 2, and 4 to facilitate comparison. We first describe results from ML/MF and AL before proceeding to AM. Fig. 1F shows the responses of three typical cells in ML/MF to both the FOB (top) and FV (bottom) image sets. Whereas Cell 1 responded strongly to the faces in the FOB set, Cell 2 responded selectively, but weakly, to faces, and Cell 3 was unresponsive. In response to the FV set, Cell 1 responded to left profiles, straight, and upward views; Cell 2 responded to left full profiles, left half profiles, straight, and upward views, and more weakly to right half profiles, downward views, and the back of the head; and Cell 3 responded only to left half and full profiles. Fig. 1G shows the responses of three typical cells from face patch AL to FOB and FV image sets. Whereas Cell 1 responded strongly to the faces in the FOB set, Cells 2 and 3 were unresponsive and suppressed, respectively. The response properties of these three AL cells thus appeared rather similar to the three ML/MF cells of Fig. 1F, when tested by FOB stimuli. In response to the FV set, Cell 1 was selective for straight, up, and downward views, Cell 2 for left and right half profiles, and Cell 3 for left and right full profiles. Thus, selectivity for head orientation of the three AL cells differed from that of the three ML/MF cells.

This difference in head orientation tuning of ML/MF and AL cells was typical for the entire population of ML/MF and AL cells. Fig. 2, A and B show the response profiles of all cells recorded in ML/MF and AL, respectively, to both the FOB (left) and FV (right) stimuli, with identical cell ordering for both plots. In AL (Fig. 2B), two distinct face view-selective populations are evident. One was excited by straight, up, and downward faces, and often suppressed by left and right

Fig. 2. Selectivity of neural populations in ML/MF, AL, and AM to faces varied in view and identity. (**A to C**) Population response matrices to the FOB image set (left) and to the FV set (right), for cells visually responsive (top) and non-responsive (bottom) in ML/MF (**A**), AL (**B**), and AM (**C**), respectively. Responses are sorted from top to bottom by the first (C) or second (A and B) principal component of the FV responses. Data combined from monkeys M1 (recordings in left hemisphere) and M3 (recordings in right hemisphere) for ML/MF, and from monkeys M1 and M2 (recordings in right hemisphere) for AL and AM. The FOB matrix for ML/MF contains only 96 elements because only the first 96 images were presented during recordings in monkey M3.



profiles (Fig. 2B, right plot, top part). When assessed by the FOB set, most of these cells proved face selective (Fig. 2B, left plot, top part). A second population responded preferentially to left and right profiles (Fig. 2B, right plot, bottom part). When assessed by the FOB set, which contained only frontal faces, many of these cells appeared not face selective (Fig. 2B, left plot, bottom part). Of these profile-selective cells, 20% were suppressed by frontal faces. In contrast to AL, in ML/MF profile-selective cells responded to only one profile view, and only 11% were suppressed by frontal-view faces. Thus, the lower incidence of face-enhanced cells in AL compared with ML/MF (Fig. 1, C and D, and fig. S2, A and B) can be explained by the specifics of head-orientation tuning in AL. Corroborating this conclusion, the majority of AL cells that appeared visually unresponsive to FOB stimuli (Fig. 2B, left plot, bottom matrix) were also profile selective (Fig. 2B, right plot, bottom matrix).

The transformation of face representation from ML/MF to AL yields a novel property in AL, not found in ML/MF: mirror symmetry of head-orientation selectivity. Not only was the de-

novo appearance of mirror symmetry in AL surprising, also surprising was the fact that such a large fraction of cells exhibited this property: 92 of 215 AL cells responded at least twice as strongly to one of the two full profiles as to frontal faces (fig. S3). These profile-selective cells responded very similarly to both profiles (the response to the nonpreferred profile view was, on average, 92% that of the preferred profile view). We next assessed the full tuning of AL cells to all head orientations. We probed 57 cells with faces randomly sampled from a three-dimensional (3D) head-orientation manifold, which was parameterized by up-down angle, left-right angle, and picture-plane angle (Fig. 3A). The faces were rendered using the face modeling software FACEGEN and refreshed at 6 Hz. To assess head-orientation selectivity, we plotted tuning along the three possible pairs of rotation dimensions while averaging over the third dimension. Pairwise head-orientation tuning profiles are shown in Fig. 3B for four example cells (the first two cells are the same as the first two in Fig. 1G). Each of the four cells showed tuning along all three head-orientation axes, and this tuning was

always mirror symmetric. Of 57 cells, 43 (75%) in the population tested had view tuning maps with two discrete peaks at mirror-symmetric positions like example cells 1 to 4.

A consequence of the emergence of mirror symmetry of head-orientation selectivity in AL is the development of partial invariance to head orientation. This can be seen in a multidimensional scaling (MDS) analysis of population response vectors (Fig. 4, A and B, and fig. S4). Although in ML/MF, each of the eight head orientations forms a discrete cluster, whose neighborhood relations reflect physical proximity of head orientation, in AL, this topological relationship is broken and responses to multiple head orientations are collapsed into joint clusters (Fig. 4B and fig. S4): Left and right full profiles are grouped into one cluster, left and right half profiles into another, and up, down, and straight into a third one.

A second difference between face representations in ML/MF and AL became apparent when we analyzed selectivity for facial identity, the second dimension of the FV stimulus set. Almost half the cells in AL were significantly modulated

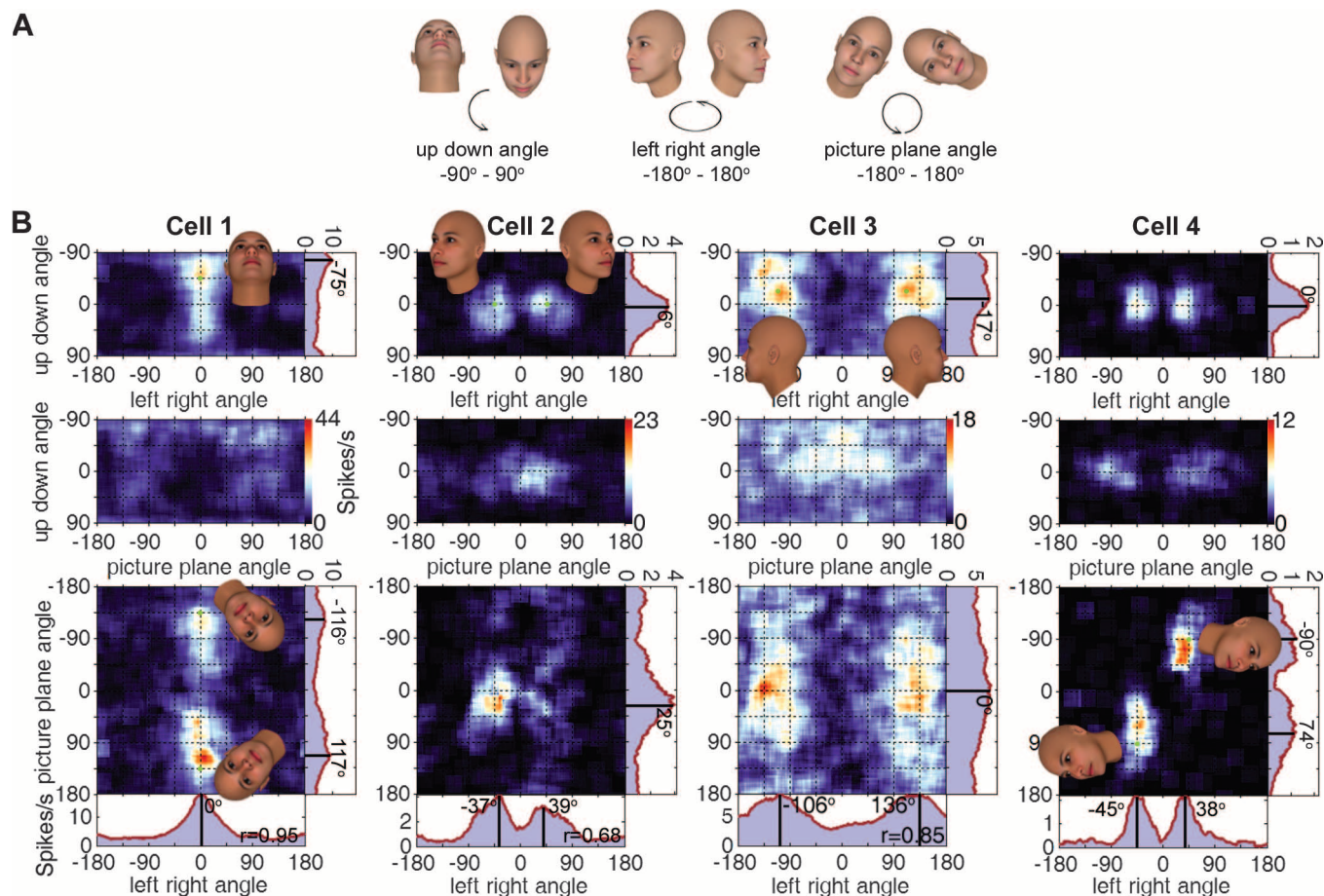


Fig. 3. Tuning of AL cells to a head randomly rotated in three dimensions. (A) Illustration of stimulus head and three axes of rotation. (B) View tuning in four typical cells. Cells 1 and 2 here are the same as Cells 1 and 2 in Fig. 1G. Cell 4 did not respond to any of the FV stimuli. (Top) Tuning to up-down angle versus left-right angle (responses averaged across picture-

plane angle). (Middle) Tuning to up-down angle versus picture-plane angle (responses averaged across left-right angle). (Bottom) Tuning to picture-plane angle versus left-right angle (responses averaged across up-down angle). Marginal tuning curves are also shown (vertical lines indicate tuning peak positions).

by facial identity (45%, analysis of variance) (fig. S5A)—significantly more than in ML/MF (19%). This is reflected in sharper identity tuning in AL compared with ML/MF (Fig. 4G and fig. S6). Because in AL partial view invariance by virtue of mirror-symmetric tuning is established, the question arises whether selectivity for facial identity generalizes across view directions in AL. We measured the similarity (correlation) between population responses to all FV images, that is, to all combinations of identity–head-orientation pairs to construct population response similarity matrices (Fig. 4, D and E) and corresponding

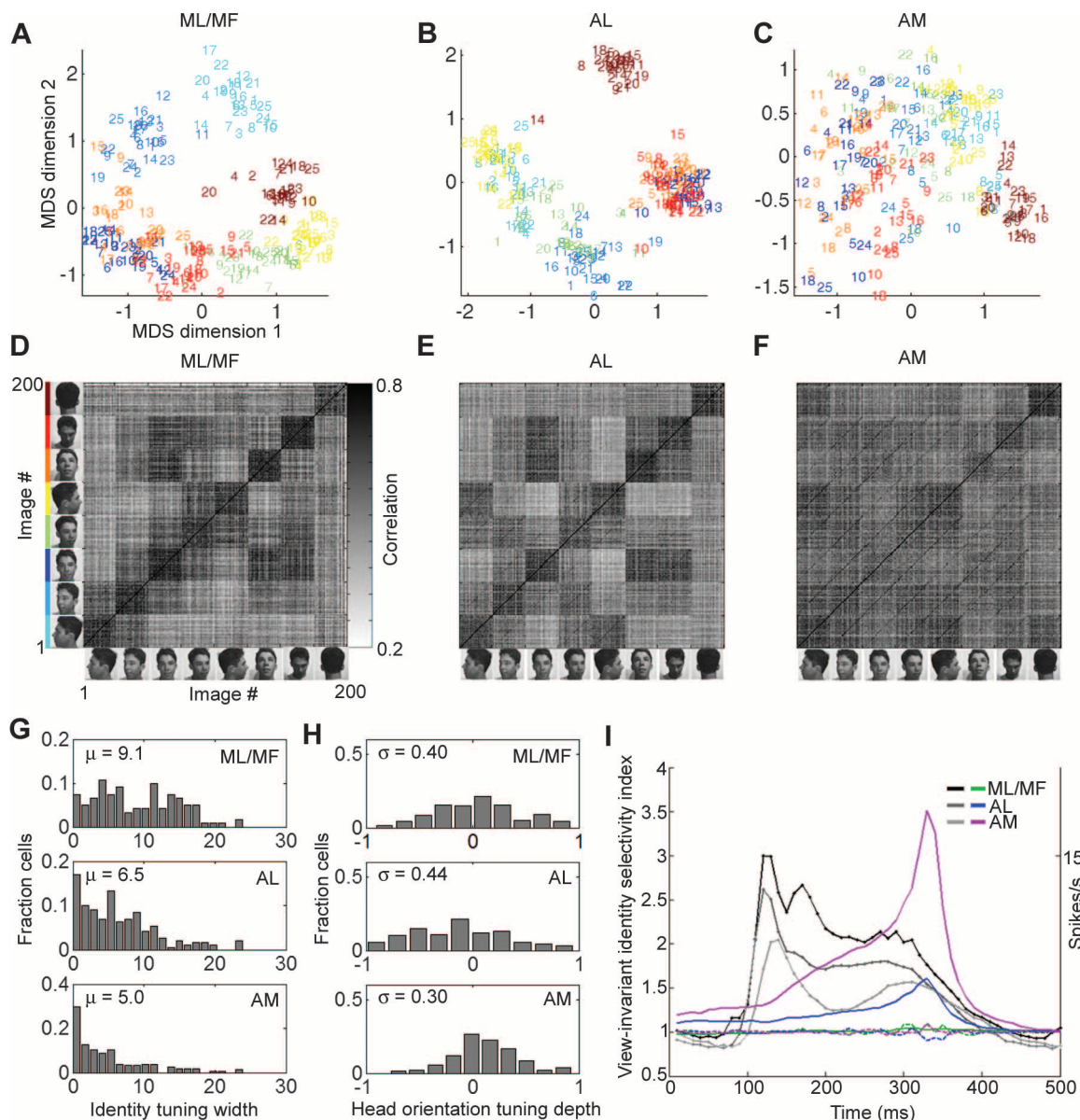
significance matrices (fig. S7, A and B). The main feature of the ML/MF similarity matrix (Fig. 4D) is high-similarity 25 by 25 squares along the main diagonal, reflecting a view-specific representation; furthermore, there are no visible paradiagonal stripes ($y = x + n \times 25$), which would emerge if population response vectors to specific individuals were similar across head orientations. The AL similarity matrix (Fig. 4E) is characterized by a different pattern of high-similarity 25 by 25 squares reflecting mirror-symmetric head-orientation tuning and by paradiagonal stripes indicating view-invariant individual selec-

tivity. These paradiagonals are not continuous but are confined to specific combinations of views (e.g., left and right full profiles). Quantification of view-invariant identity selectivity in the populations of ML/MF and AL cells is shown in fig. S5, B and C. View-invariant identity tuning was significantly stronger in AL than in ML/MF (fig. S5D).

AM, like AL, showed a different pattern of face selectivity than ML/MF when probed with FOB stimuli (Fig. 1E and fig. S2C). Again, the FV stimuli revealed the cause. Fig. 1H shows the responses of three AM example cells to the FOB

Fig. 4. Population representations of face view and identity in ML/MF, AL, and AM.

(A to C) Comparison of multidimensional scaling plots of responses to the FV image set in ML/MF, AL, and AM. Each plot shows the location of the 25 faces (indicated by numbers 1 to 25) at eight head orientations [indicated by eight colors, key in (D)] within the first two dimensions of the MDS space (corresponding Eigenvalues in fig. S3). (D to F) Population similarity matrices in the three face patches. A 200 by 200 matrix of correlation coefficients was computed between responses of all visually responsive cells to the 200 FV stimuli from ML/MF ($N = 121$ cells), AL ($N = 189$ cells), and AM ($N = 158$ cells). The correlation patterns do not change when only the first 121 cells of each patch are considered (fig. S13). (G) Sharpness of identity tuning in ML/MF, AL, and AM (top to bottom). Central tendencies of distributions of identity tuning half-widths (see SOM) were significantly different from each other ($P < 0.001$, Mann-Whitney U tests). (H) Distributions of head-orientation tuning depths (see SOM). Tuning depths close to 0 indicate broad tuning. These distributions are significantly different from each other ($P < 0.001$ for ML/MF versus AL and AL versus AM, Mann-Whitney U tests; $P < 0.002$ for ML/MF versus AM and AL versus AM, F test). (I) Evolution of view-invariant identity selectivity over time. View-invariant identity selectivity index, computed over a 200-ms sliding response window beginning at the indicated time point, plotted for AM, AL, and ML/MF (solid curves). The dotted



curves show the mean view-invariant identity-selectivity index over time computed from shuffled similarity matrices. The grayscale traces show the time course of the mean response to the FV stimuli across the population in each face patch. The substantial delay between the peak of the mean response to the FV stimuli and the peak of the view-invariant identity-selectivity index suggests that recurrent mechanisms are involved in the computation of view-invariant identity.

and FV stimuli. Cell 1 responded strongly to the FOB faces and to all nonbackward head orientations in the FV set. Cells 2 and 3 were unresponsive to all images in the FOB set, and each responded strongly to only two individuals in the FV set, across almost all nonbackward views. These example cells span the range of responses we observed across the population (Fig. 2C). From the population response, two new characteristics of face representations in AM, found neither in ML/MF nor in AL, are apparent. First, cells in AM were far less sharply and strongly tuned to head orientation than those in AL or ML/MF (Fig. 4H). This is reflected in the MDS analysis (Fig. 4C), which shows that in AM, unlike ML/MF and AL, population responses were only weakly organized by head orientation. Second, AM cells spanned a wide range of identity selectivity from complete lack of identity tuning to sharp identity tuning; in particular, we observed cells like Cells 2 and 3 in Fig. 1H with sparse, individual-specific, view-invariant response patterns only in AM. The specificity of some AM cells was so extreme that they did not respond to any of the faces in the FOB set, explaining the lower apparent face selectivity of AM compared with ML/MF. Even sparser cells may exist in AM, cells that do not respond to any of the FOB or FV stimuli.

Overall, an even larger fraction of cells in AM than in AL were tuned to facial identity (73% versus 45%) (fig. S5A), and tuning to facial identity was sharper in AM than in AL (Fig. 4G). How does identity selectivity depend on head orientation in AM? The population similarity matrix for AM (Fig. 4F and fig. S7C), unlike those for ML/MF and AL, exhibited only a weak dependence on head orientation; rather, its main feature was robust paradiagonal stripes across all head orientations, except the back of the head. For example, the population response to individual 7 at left full profile was more similar to the response to the same individual at upward head orientation than to the response to individual 12 at left full profile. Quantification of view-invariant identity selectivity (figs. S5, B and C) shows a further increase in AM, compared with ML/MF and with AL; both differences are highly significant (fig. S5D). Thus, response similarity in AM is abstracted from pixel-wise picture similarity: The population of AM cells approaches a view-invariant representation of facial identity. It may be surmised that view-invariant identity in AM is carried primarily by sparsely firing cells. Yet we found that both sparsely and nonsparingly responding cells contained view-invariant identity information (fig. S8).

Although we have emphasized feed-forward transformations between face patches as the major change in face representation, processing within face patches and recurrent processing between patches at different levels of the processing hierarchy are likely further mechanisms that, over time, bring about more elaborate representations (15). Indeed, view-invariant face selectivity as

assessed by the similarity matrices changed substantially over the course of time in a manner not directly reconcilable with a simple, one-time feed-forward mechanism of transformation (Fig. 4I). Rather, the buildup over time suggests additional recurrent mechanisms. Only representations in AM and AL, but not ML/MF, profited from the passage of time to increase identity selectivity in a view-invariant manner (Fig. 4I). Thus, whatever mechanism brings about view-invariant identity selectivity does not indiscriminately involve all face patches but only specific subsets.

Different schemes for processing facial information have been proposed. In particular, it has been suggested that after an early encoding stage two parallel streams emerge (16), a dorsal one in the superior temporal sulcus (STS) related to coding changeable aspects of faces, and a more ventral one coding structural aspects of faces (17). Applied to the macaque brain, this scheme implies a parallel arrangement of AL and AM. Alternatively, the three face patches could be arranged in a hierarchical manner. Thus, the transformation that gives rise to an almost fully view-invariant representation in AM may originate directly from ML/MF (in parallel to the transformation occurring between ML/MF and AL) or mostly from AL (fig. S9A). The increase in invariance to head orientation from ML/MF to AL and further to AM (Fig. 4, A to F, and figs. S5 and S7) suggests a hierarchical arrangement. Also, consistent with AM receiving inputs from AL, the population similarity matrix for AM showed residual traces of mirror symmetry (Fig. 4F). A separate experiment in which we tested for invariance to spatial position showed a similar increase of invariance from ML/MF to AL, and further to AM (fig. S10). Independent and more direct evidence for parallel or hierarchical arrangements can be derived from the relative timing of neural responses across face patches. First, we consider local field potential (LFP) responses (fig. S11). In all three patches, the LFP evoked by faces was clearly distinct from that evoked by nonface objects: The latency of the first peak to faces was at least 12 ms shorter than that to nonface objects. The latencies of the first face-evoked response peaks increased from ML/MF (126 ms), to AL (133 ms), and further to AM (145 ms). This sequential increase is most easily reconciled with a hierarchical arrangement of the face patches. If a major direct projection from ML/MF to AM existed without additional synaptic relays, one would expect that a first activity wave would be triggered in AM at approximately the same time as that in AL, not at more than twice the ML/MF-AL delay. Second, response latencies of neurons in the three face patches (fig. S12) systematically increased from ML/MF (average 88 ms) to AL (104 ms) and further to AM (124 ms). Again, this is difficult to explain without assuming additional steps of synaptic transmission between ML/MF and AM compared with ML/MF and AL, and the most parsimonious explanation would be relay through AL.

The finding that view dependence of identity tuning was fundamentally different in the three face patches (Fig. 4, A to F) addresses a fundamental problem for computational neuroscience (18): How can increasingly shape-selective but otherwise invariant representations be generated? Our results suggest that pooling across mirror-symmetric views may constitute a crucial step in this process. Fig. S9B presents a sequential model for establishing a view-invariant representation of faces, motivated by the response properties of neurons recorded in ML/MF, AL, and AM. At the first level (view-specific, I), each neuron responds most strongly to a specific individual at a specific view. At the next level (partially view-invariant, II), each neuron pools together inputs representing mirror-symmetric views of the same individual (left and right full profiles, green axons; left and right half profiles, blue axons), or straight, upward, and downward views of the same individual (red axons), with lateral inhibition between profile- and frontal-selective neurons, and between neurons representing different identities. Finally, in a view-invariant stage (level III), each neuron pools together inputs representing all views of the same individual, with lateral inhibition to generate sparse responses. The model predicts similarity matrices (fig. S9C), which resemble the ones found in the three face patches (Fig. 4, D to F), capturing their main features of view tuning (square structures) and view-invariant identity selectivity (paradiagonal stripes).

The greatest obstacle to object recognition is the huge amount of variation that can occur in the retinal images cast by a 3D object. Our finding of individual-selective responses with a high degree of invariance across head orientations in AM was obtained with an image set containing faces never encountered in real life. Thus, whatever learning has occurred before the experiments, it has resulted in a face representation that allows generalization of selectivity for new faces. The face system may already incorporate all a priori knowable invariances of a bilaterally symmetric 3D object, a face, into a canonical face space (19–21), such that for an a posteriori encountered novel face, a largely invariant response can be generated without necessity for further learning. Although experience with an actual individual is not a necessary condition for representations in AM, such experience may yield an even more invariant representation (22–24).

Four results provide new insights into the functional organization of the macaque face-patch system. First, all face regions recorded from contained a high degree of face-selective neurons (lower estimates using only the FOB stimulus set: 97% in ML/MF, 86% in AL, and 89% in AM). Extrapolating from these findings, it seems likely that the entire network of temporal lobe face patches constitutes a dedicated brain system for the processing of one high-level object-category, faces. Second, structure and function are highly correlated. A face patch at a particular anatomical location harbors a specific face representation

that is qualitatively different from the face representation in a face patch at a different location, and further differences are likely to exist between face representations in the different face patches. Thus, attaching a name to a given face patch is now shown to be meaningful because it signifies functional identity across individuals. Together with earlier results (12, 13, 25), this shows that the face-processing system is a network composed of multiple, functionally specialized nodes. Third, the system contains one region (AM) that provides for population coding of identity across view conditions, using a hybrid representation of both coarse and sparse elements (26–28). Fourth, the finding of an entire face patch, yet only one, containing neurons with mirror-symmetric tuning is worth particular emphasis because it raises the possibility that such a representation constitutes a critical computational step for object recognition. Even though inferotemporal cells with mirror-symmetric tuning have been reported before (29, 30), this is the first time that such cells have been shown to be agglomerated within a single intermediate node within a form-processing network. Why would this step be useful? One possibility, following earlier work on view-tuned face-selective neurons in the STS (7, 31–33), is that this stage serves to extract socially important information from head orientation, because face (and gaze) aversion carries similar social meaning whether for leftward or rightward orientation. Alternatively, such a stage may provide computational advantages for efficient coding (34). Why are the three view stages of the face-processing system located in separated regions and not next to each other? One possibility is that the face-processing system interdigitates with representations for other objects implementing the same

three view stages. The pattern of viewpoint generalization revealed here for faces may thus turn out to be a general organization principle of the entire inferotemporal cortex.

References and Notes

1. I. Biederman, *Spat. Vis.* **13**, 241 (2000).
2. C. Bruce, R. Desimone, C. G. Gross, *J. Neurophysiol.* **46**, 369 (1981).
3. D. I. Perrett, E. T. Rolls, W. Caan, *Exp. Brain Res.* **47**, 329 (1982).
4. E. T. Rolls, *Hum. Neurobiol.* **3**, 209 (1984).
5. R. Desimone, T. D. Albright, C. G. Gross, C. Bruce, *J. Neurosci.* **4**, 2051 (1984).
6. S. Yamane, S. Kaji, K. Kawano, *Exp. Brain Res.* **73**, 209 (1988).
7. M. E. Hasselmo, E. T. Rolls, G. C. Baylis, V. Nalwa, *Exp. Brain Res.* **75**, 417 (1989).
8. K. Tanaka, H. A. Saito, Y. Fukada, M. Mori, *J. Neurophysiol.* **66**, 170 (1991).
9. N. Kanwisher, J. McDermott, M. M. Chun, *J. Neurosci.* **17**, 4302 (1997).
10. D. Y. Tsao, W. A. Freiwald, T. A. Knutsen, J. B. Mandeville, R. B. Tootell, *Nat. Neurosci.* **6**, 989 (2003).
11. M. A. Pinsk, K. Desimone, T. Moore, C. G. Gross, S. Kastner, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 6996 (2005).
12. D. Y. Tsao, S. Moeller, W. A. Freiwald, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 19514 (2008).
13. S. Moeller, W. A. Freiwald, D. Y. Tsao, *Science* **320**, 1355 (2008).
14. K. S. Saleem, K. Tanaka, K. S. Rockland, *Cereb. Cortex* **3**, 454 (1993).
15. Y. Sugase, S. Yamane, S. Ueno, K. Kawano, *Nature* **400**, 869 (1999).
16. V. Bruce, A. Young, *Br. J. Psychol.* **77**, 305 (1986).
17. J. V. Haxby, E. A. Hoffman, M. I. Gobbini, *Trends Cogn. Sci.* **4**, 223 (2000).
18. M. Riesenhuber, T. Poggio, *Nat. Neurosci.* **2**, 1019 (1999).
19. T. Valentine, *Q. J. Exp. Psychol. A* **43**, 161 (1991).
20. D. A. Leopold, A. J. O'Toole, T. Vetter, V. Blanz, *Nat. Neurosci.* **4**, 89 (2001).
21. E. McKone, A. Aitkin, M. Edwards, *J. Exp. Psychol. Hum. Percept. Perform.* **31**, 1181 (2005).
22. Y. Miyashita, *Nature* **335**, 817 (1988).
23. G. Wallis, H. H. Bülthoff, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4800 (2001).
24. N. Li, J. J. DiCarlo, *Science* **321**, 1502 (2008).
25. D. Y. Tsao, W. A. Freiwald, R. B. H. Tootell, M. S. Livingstone, *Science* **311**, 670 (2006).
26. H. B. Barlow, *Perception* **1**, 371 (1972).
27. M. P. Young, S. Yamane, *Science* **256**, 1327 (1992).
28. E. T. Rolls, *Neuropsychologia* **45**, 124 (2007).
29. J. E. Rollenhagen, C. R. Olson, *Science* **287**, 1506 (2000).
30. N. K. Logothetis, J. Pauls, T. Poggio, *Curr. Biol.* **5**, 552 (1995).
31. D. I. Perrett et al., *Proc. R. Soc. Lond. B Biol. Sci.* **223**, 293 (1985).
32. D. I. Perrett et al., *Exp. Brain Res.* **86**, 159 (1991).
33. S. Eifuku, W. C. De Souza, R. Tamura, H. Nishijo, T. Ono, *J. Neurophysiol.* **91**, 358 (2004).
34. T. Vetter, T. Poggio, H. H. Bülthoff, *Curr. Biol.* **4**, 18 (1994).
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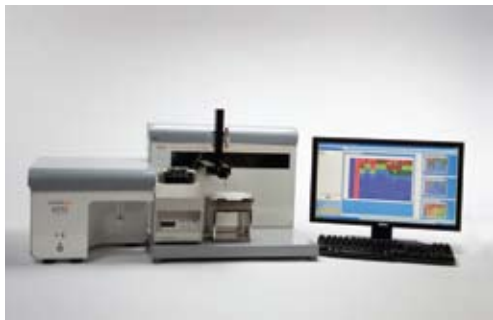
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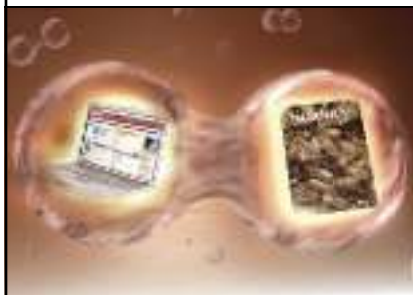
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Interested candidates should submit applications electronically as a single PDF file (titled last name, first name) to **e-mail: sf35@cornell.edu**. The file should include curriculum vitae, contact information for three references, a statement of future research interests, and cover letter addressed to **Dr. Ted Clark**, Faculty Search Committee Chair, Department of Microbiology and Immunology.

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Cornell University is an Affirmative Action/Equal Opportunity Employer and Educator. Cornell University embraces diversity and seeks candidates who will create a climate that attracts students of all races, nationalities, and genders. Women, under-represented minorities, and people with disabilities are strongly encouraged to apply.



LECTURER IN BIOLOGICAL SCIENCES The University of Chicago Biological Sciences Collegiate Division

The Biological Sciences Collegiate Division at the University of Chicago invites applications for a non-tenure-track three-quarter Lectureship in molecular/developmental biology beginning in the winter of 2011. The Lecturer is expected to co-direct the fall and winter laboratory courses for Biology majors and develop a new module for Core Biology for non-majors in the spring quarter. Applicants must have received a Ph.D. in Biology or related subspecialties, have at least three years of postdoctoral research experience and must document interest and proficiency in undergraduate teaching. The appointment will be for three years and is renewable. To apply visit [website: https://academiccareers.uchicago.edu](https://academiccareers.uchicago.edu), select **requisition #00608**, and upload your cover letter, curriculum vitae, teaching statement, research statement, and provide contact information for your three reference providers. Your application and supporting materials must be uploaded by December 13, 2010.

The University of Chicago is an Affirmative Action/Equal Opportunity Employer.

Professor of Neuro Imaging



qbi
queensland brain institute

cai
centre for advanced imaging

**Queensland Brain Institute (QBI) and the
Centre for Advanced Imaging (CAI)**
in Brisbane, Australia

QBI (www.qbi.uq.edu.au) and the newly established CAI (www.uq.edu.au/cai) seek a creative, accomplished Group Leader with an internationally recognized research program in neuroimaging.

Applicants should have an established reputation and academic leadership in the area of neuroimaging with a strong interest in the development of image analysis methods, neuroimaging in small animal models and/or translational applications to the human nervous system.

The appointment will be at Professorial level E at the University of Queensland and a competitive start-up package will be provided.

QBI's principal research themes target brain plasticity and include Cellular plasticity, Synaptic plasticity, Cognitive and behavioural neuroscience, Visual neuroscience, Mental and neurological disorders and Imaging and computational neuroscience.

The Institute is housed in a \$63 million facility on the campus of the University of Queensland, fitted with state-of-the-art research equipment. The Institute currently accommodates some 320 scientists, students and support staff. QBI has access to a range of worldclass technologies, including a 16.4T small animal scanner, 4.6T MRI scanner and a 3T human research MRI. It also has extensive capabilities in animal and human behavioural testing, and has developed strong interdisciplinary teams in the area of applying nanotechnology to neuroscience.

CAI is a newly created University Centre whose overall goal is to create a world-class integrated facility for research and training in biomedical imaging. This state-of-the-art facility will provide an integrated, multidisciplinary and multimodal research framework for basic, translational and clinical research in biomedical imaging with research ultra high-field (7T) human MRI, PET, MRI/PET and radiochemistry facilities.

The University of Queensland is the lead institution of the National Imaging Facility.

QBI and CAI are developing a collaborative network in the Asia-Pacific region and has recently signed affiliation agreements with leading neuroscience and imaging institutes in the Asia-Pacific region.

To apply please send a cover letter, a CV, a three page statement of research interests, and arrange to have 3 letters of recommendation sent to the Search Committee.

Applications should be sent either by email to h.weir@uq.edu.au or by regular mail to Ms Helen Weir, Queensland Brain Institute, The University of Queensland, St Lucia, QLD 4072, Australia.

Applications should be received by Friday 28 January 2011 to assure full consideration.

Postdoctoral Positions in Synaptic Molecular Mechanisms and Optogenetic Circuit Analysis

The Center for Functional Connectomics (CFC) is recruiting postdoctoral fellows worldwide to join our dynamic new research group. The CFC is located at the Korea Institute of Science and Technology (KIST) and consists of a number of outstanding laboratories focused on the following research areas:

Molecular mechanisms of synaptic transmission

- George Augustine** (georgea@neuro.duke.edu) – exocytosis and endocytosis in presynaptic terminals; gene expression changes during long-term synaptic plasticity
Jinny Kim (kimj@janelia.hhmi.org) – local protein synthesis; ion channel trafficking in dendrites
Eunmi Hwang (emhwang@kist.re.kr) and **Jae-Yong Park** (jaeyong@gsnu.ac.kr) – ion channel regulation and trafficking in neurons and astrocytes
Justin Lee (cjl@kist.re.kr) – neuron-glial signaling mechanisms
Mikyong Park (mpark3@stanford.edu) – synaptogenesis and synapse elimination during synaptic plasticity
Keiko Tanaka (keiko@kist.re.kr) – signaling during long-term synaptic plasticity

Optogenetic analysis of brain circuitry

- George Augustine** – high-throughput connectome mapping
Larry Cohen (lawrence.cohen@yale.edu) – development of optogenetic voltage-sensor proteins; imaging circuit activity in the olfactory system
Homme Hellinga (hwh@biochem.duke.edu) – protein design; optogenetic probe engineering
Jinny Kim – mapping brain circuitry with GRASP technology
Justin Lee – optogenetic mapping of neuron-glial signaling pathways
Sebastien Royer (royers@janelia.hhmi.org) – silicon probes; optogenetic studies of hippocampal place cell circuitry

In addition, the CFC includes a network of secondary faculty and collaborators who also will serve as excellent postdoctoral mentors; see our website <http://cfc.wci.re.kr/english/portal.php>.

Beyond exciting research projects and excellent mentors, the CFC offers our postdocs a number of other benefits:

- stimulating and highly interactive scientific environment
- state-of-the-art research facilities
- strong and secure funding through the World Class Institute program, which recruits foreign scientists to enrich the scientific environment of Korea
- high pay (NIH scale)
- free housing
- living in Seoul, one of the world's largest and most exciting cities

Candidates should have a PhD or MD degree (or should be planning to graduate soon) and should be comfortable speaking English, the primary language of the CFC. Applicants should send their curriculum vitae and a list of potential referees via e-mail, either directly to the relevant faculty member or to the CFC (cfc@kist.re.kr).

Life Inspired.

Genentech Research and Early Development – Neuroscience



Mara, Patient

For more than 30 years, Genentech has been at the forefront of the biotechnology industry, using human genetic information to develop novel medicines for serious and life-threatening diseases. Now a member of the Roche Group, Genentech has multiple therapies on the market for cancer and other serious illnesses.

Genentech's Research and Early Development (gRED) department operates as an independent center within the Roche Group and is home to the largest single-site biotech research facility in the world. gRED explicitly fosters individual creativity and initiative among its researchers, encouraging scientists to pursue projects of interest in addition to working toward the company's goals. As a result, our more than 1,100 scientists and researchers and 125 postdocs have consistently published important papers in prestigious peer-reviewed journals and are among the top researchers in the world in terms of total citations. gRED combines the best of the academic and corporate worlds, allowing researchers not only to pursue important scientific questions, but also to watch an idea move from the laboratory into clinical development.

We invite candidates for the following opportunities in our South San Francisco, CA, headquarters:

Scientists – Requisition #1000034748

Senior Research Associates – Requisition #1000034264

Research Associates – Requisition #1000034394

Consistently recognized as one of the top companies to work for in the United States, Genentech is dedicated to remaining a great place to work and to providing employees with programs, services and benefits that allow them to bring the best to the business and to their personal lives. For a complete job description and to learn more about our current opportunities, please visit careers.gene.com and reference the Requisition #. Use "Ad-Science" when a source is requested. Genentech is an equal opportunity employer.

In October 2010, Genentech was named "top employer in the biopharmaceutical industry" by Science magazine.



What does "Life Inspired" mean to you?

"Diseases of the brain such as Alzheimer's disease and schizophrenia cause immeasurable suffering to countless people throughout the world. Effective treatments can only be discovered by excellent innovative science. For me, "Life Inspired" represents the dedicated scientists at Genentech who come to work every day eager to apply their expertise, creativity and team spirit to making a difference for patients."

Morgan Sheng, Ph.D.

Vice President, Neuroscience
Two years with Genentech



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A Member of the Roche Group



UNIVERSITY OF
BIRMINGHAM



Understanding the brain

Centre for Computational Neuroscience and Cognitive Robotics

A new Centre for Computational Neuroscience and Cognitive Robotics (CN-CR, www.psychology.bham.ac.uk/cncr) has been launched, with a multimillion pound investment. The Centre combines research on human cognition, sensory and motor systems, and computational modeling with research on robotic systems leading towards a better understanding of both brain function and advanced robotics. The research will also be translated into innovative treatments for brain injuries and those with degenerative or developmental neurological disorders.

Chair in Computational Neuroscience (1 or 2 posts)
Competitive package for an outstanding candidate.
(Ref: 38207)

You should have an international research record and be making a cutting-edge contribution to the field.

Senior Lectureships or Lectureships in Computational Neuroscience (2 posts)
Salary from £45,155 to £68,302 a year or from £36,715 to £49,342 a year (Ref: 47252)

You will have a growing reputation in the field, a strong publication record and the potential to become a world leader.

Informal enquiries: Professor Glyn Humphreys (0121 414 4930; g.w.humphreys@bham.ac.uk), Professor Chris Miall, Head of School of Psychology (0121 414 2867; r.c.miall@bham.ac.uk) and/or Dr Jeremy Wyatt, School of Computer Science (0121 414 4788; j.l.wyatt@bham.ac.uk)

To download the details and submit an electronic application online visit: www.hr.bham.ac.uk/jobs

Closing date for all posts: 10 December 2010

www.hr.bham.ac.uk/jobs

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Tufts
UNIVERSITY

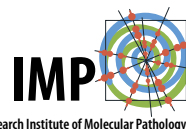
School of Medicine

Faculty Positions in Neuroscience

The Department of Neuroscience (www.neurosci.tufts.edu) at Tufts University School of Medicine is expanding by adding tenure-track faculty positions. Positions are available at **Assistant, Associate and Full Professor** levels. The department will build on its core strengths and focus on the study of synapses, disorders of the nervous system and neuron-glia interactions. We are particularly interested in building on our research that is relevant to epilepsy, depression, neurodegenerative disorders and the neurobiology of obesity. We are seeking candidates who use innovative approaches to investigate problems that cross levels of investigation from molecular and cellular to systems and/or behavioral neuroscience. Candidates using molecular, genetic, electrophysiological and/or imaging methodologies to study neurons, synapses and networks are particularly encouraged to apply. We offer generous startup packages, newly renovated laboratory space and a highly collaborative environment offering opportunities for both basic and translational research.

Applicants should hold a Ph.D. and/or M.D. degree and have several years of productive postdoctoral experience. Successful candidates will be expected to develop thriving, well-funded research programs and to contribute to graduate and medical education. Please submit electronic applications including a CV, a statement of research interests and the names and e-mail addresses of at least three references to: neurosci-facultyrecruitment@tufts.edu.

*TUSM is an Equal Opportunity Affirmative Action Employer.
Women and minorities are encouraged to apply.*



PhD and Postdoctoral Positions Neural Circuits and Behavior

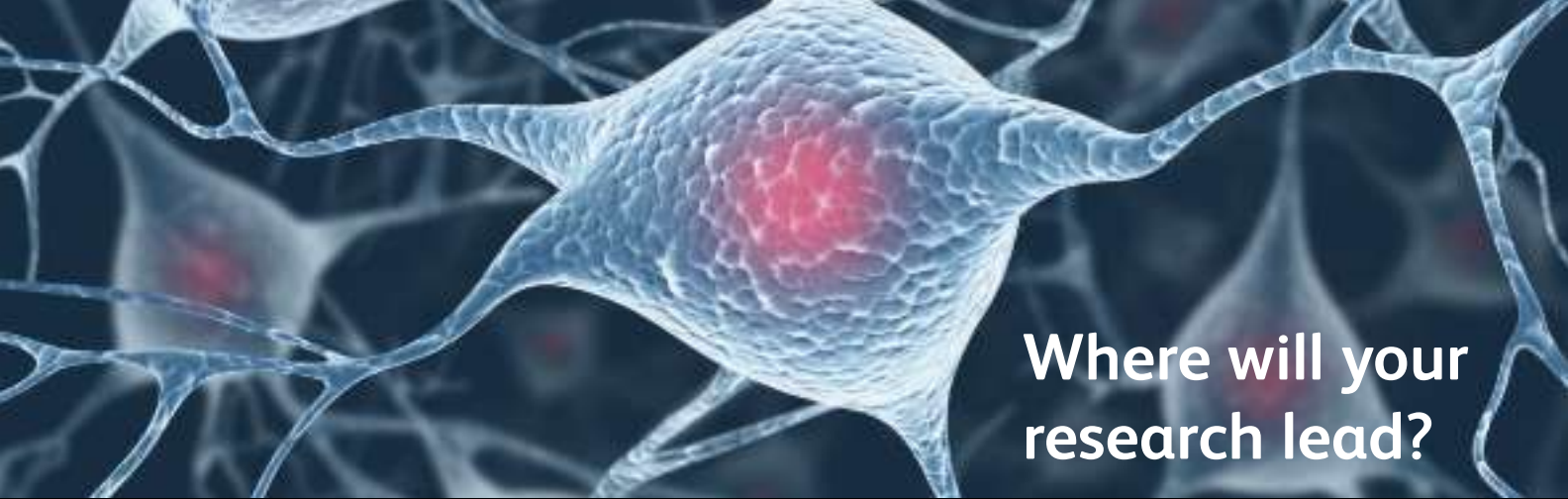
The Research Institute of Molecular Pathology (IMP) has recently expanded its neuroscience research community to establish a strong interdisciplinary focus on the functional analysis of neural circuits underlying innate and learned behaviors in genetic model organisms. Positions for PhD students and postdoctoral researchers are now available in the following groups:

Barry Dickson - Mating and other behaviors in *Drosophila*
Wulf Haubensak - Fear circuits in mice
David Keays - Molecular and cellular basis of magnetoreception
Krystyna Keleman - Courtship learning in *Drosophila*
Simon Rumpel - Auditory processing and memory in mice
Andrew Straw - Visual guidance of *Drosophila*
Alipasha Vaziri - Advanced optogenetic technologies
Manuel Zimmer - Olfactory behaviors in *C. elegans*

The IMP is a privately sponsored basic research institute located near the center of Vienna, one of the world's most cosmopolitan, attractive, and affordable cities. The neuroscience community is complemented by other research groups in computational biology, molecular and cell biology, immunology and oncology, and is supported by outstanding core services. Students and postdocs from more than 40 different nationalities work at the IMP. English is the working language throughout the institute.

For further details on the research activities and open positions in each group, contact the respective group leader. Applicants should be passionately committed to understanding brain functions and have a strong background in any relevant field, including neuroscience, molecular and cell biology, genetics, computer science, physics, engineering, and/or mathematics. PhD students will take part in the VBC international PhD program, which has its next deadlines for applications on November 15, 2010 and April 30, 2011.

See: www.imp.ac.at/neuroscience for more information.



**Where will your
research lead?**

PFIZER POSTDOCTORAL PROGRAM IN NEUROSCIENCE

The Pfizer Neuroscience Research Unit announces the creation of the Pfizer Neuroscience Postdoctoral Fellowship Program. Based in Groton, Connecticut, the Pfizer Neuroscience Fellowship Program provides an unparalleled opportunity for young scientists interested in understanding brain disorders and translating knowledge into innovative medicines. We seek motivated and enthusiastic individuals with a demonstrated track record in basic or translational neuroscience interested in pursuing an academic-style post-doc in an industry setting. Successful candidates will come from a range of backgrounds including molecular/cellular biology, protein biochemistry, in vitro and in vivo electrophysiology, imaging, neurochemistry, systems neuroscience, computational biology, animal models of disease, synapse biology and behavioral/cognitive neuroscience.

Postdoctoral positions are available in the following disease areas:

Alzheimer's disease Autism Bipolar Disorder Parkinson's disease Schizophrenia

Postdoctoral fellows will gain access to the resources, experience and collaborative industrial-academic network available within the Neuroscience Research Unit, conduct independent research, publish high profile papers on cutting edge areas of neuroscience, and attend and present at international scientific meetings. At the same time, postdoctoral fellows will contribute to Pfizer's commitment to deliver new innovative medicines that effectively treat neurological and psychiatric diseases.

Interested candidates can explore and apply for these competitive positions at
www.PfizerNeuroscience.com.

Applications are received and reviewed in the spring and fall. The deadline for spring applications is April 1st and for fall applications is October 1st. Fellowships are extremely competitive. We anticipate inviting a small number of candidates to interview in May and November at the Pfizer Groton Research Laboratories.

We are proud to be an equal opportunity employer and welcome applications from people with different experiences, backgrounds and ethnic origins.



Working together for a healthier world™



TEXAS TECH UNIVERSITY



TEXAS TECH UNIVERSITY
HEALTH SCIENCES CENTER

Professors of Neuroscience - Imaging

Texas Tech University and the Texas Tech University Health Sciences Center have collaborated to expand research in neuroscience with an emphasis on multidisciplinary research. Two full professor positions are available and will be based at Texas Tech University and involve joint appointments with the Texas Tech University Health Sciences Center. A new 4,000 sf core facility with a Siemens 3-T MRI with functional and diffusion tensor capabilities and 4,000 sf of support offices and laboratories is available. The footprint includes space for 2 additional fully capable MRIs in the core facility. Candidates will be in any Biological Science or Engineering department including but not limited to Electrical Engineering, Biological Sciences, Psychology, and Human Development and Family Studies. Support from other core facilities is available in biotechnology, genomics and high performance computing. Both of these positions are intended to add critical mass to our new Neuroimaging Institute and successful applicants will be expected to work with current faculty to grow this institute. A separate search will be initiated to find a director for this institute. We are particularly interested in building on our strengths in neural and behavioral biology including but not limited to drug and alcohol addiction, autism, aging, and brain development. Other research areas of functional imaging outside of neuroscience may be considered.

Applicants will be expected to have significant competitive grant funding and a clear record of scholarship, research productivity, mentoring, team building, outreach, and teaching. Multidisciplinary research across academic departments is encouraged. Graduate advising and teaching is expected in areas of interest to the faculty. Service to the institutions and the profession are important aspects of a successful applicant. Level of academic appointment is open to all ranks, but preference will be given to individuals who qualify for full professor or already are at that rank.

Screening will begin upon the receipt of applications and will continue until both positions are filled. Applicants must submit a letter of interest expressing their vision for research contributions and graduate education goals, a complete resume/CV, the names of five references who may be contacted to provide a professional assessment of research and graduate education capabilities, and start-up requirements. These should be submitted electronically to <http://jobs.texasstate.edu>; search postings for requisition **82516**. Questions about the Neuroscience-Imaging positions should be directed to: **Taylor Eighmy, Ph.D., Vice President for Research, Texas Tech University, Box 41075, Lubbock, Texas 79409-1075; taylor.eighmy@ttu.edu; 806.742.3905.**

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UNIVERSITY OF
ROCHESTER
MEDICAL CENTER

FACULTY POSITION NEUROSCIENCE

The Center for Neural Development and Disease at the University of Rochester School of Medicine and Dentistry invites applications for a tenure-track faculty position at the Assistant, Associate, or Full Professor level. Candidates studying nervous system development and function using genetic approaches in vertebrate or invertebrate model systems are particularly encouraged to apply. Though not a prerequisite, a record of success in obtaining extramural research support is an asset.

The Center for Neural Development and Disease is a dynamic, interdepartmental group of investigators whose research spans a broad area of molecular and cellular neuroscience. Members of the Center hold faculty appointments in a variety of basic science and clinical departments, including Neurology and Biomedical Genetics, allowing multiple opportunities for interaction and collaboration.

Interested candidates should submit a cover letter, CV, and statement of research interests to cnddsearch@urmc.rochester.edu. Please also arrange for three letters of reference to be sent to this address. Review of applications will begin immediately.

The University of Rochester is an Equal Opportunity Employer, has a strong commitment to diversity and actively encourages applications from candidates from groups underrepresented in higher education.



MIAMI
UNIVERSITY
OXFORD OHIO

PHYSIOLOGY/NEUROSCIENCE

Miami University is seeking to fill a tenure-track assistant professor position in an area that will complement research activities of the faculty. The successful applicant will be expected to maintain an active research program, acquire extramural funding, supervise student research, and participate in teaching graduate and undergraduate courses. In addition, s/he will participate in the new interdepartmental Cellular, Molecular, and Structural Biology PhD program as well as interdisciplinary minors in neuroscience and/or molecular biology. The University has state-of-the-art facilities for using molecular, cellular and whole organism techniques and offers competitive start-up funds. The Department has 32 faculty, over 60 PhD/MS students, and approximately 1,000 majors (<http://zoology.muohio.edu/>). Miami University (enrollment 24,000) is rated nationally as a highly selective public university.

Send letter of application, curriculum vitae, statements of teaching and research interests, and three letters of recommendation to: **Dr. Paul James, Search Committee Chair, Department of Zoology, 212 Pearson Hall, Miami University, Oxford, OH 45056**. Review of applications will begin on **7 December 2010** and continue until the position is filled. PhD required. Telephone **513-529-3100** or e-mail biosearch@muohio.edu for more information. For information regarding campus crime and safety, visit www.muohio.edu/righttoknow. Hard copy upon request.

*Miami University is an EOE/AA Employer
with smoke-free campuses.*

Call for Nominations

8th Annual Edward M. Scolnick Prize in Neuroscience
The Edward M. Scolnick Prize in Neuroscience recognizes an outstanding discovery or significant advance in the field of neuroscience. The Prize consists of US \$60,000. The recipient presents a public lecture at MIT, hosted by the McGovern Institute.

Nominating Procedures: Self-nomination is not allowed. Candidates for the award are to be nominated by individuals affiliated with universities, hospitals, medical schools, and research institutes with a background in neuroscience. Each nomination should include a) a biosketch or CV of the nominee, and b) a letter of nomination (which includes a summary and analysis of the major contributions of the nominee to the field of neuroscience). Up to two representative reprints will be accepted. The winner must be able to attend all events to be awarded the prize.

Deadline: The nomination and supporting documentation must be received no later than November 30, 2010. Members of the selection committee and faculty affiliated with MIT are not eligible. Announcement of the award recipient will be made in February 2011.

Previous Winners: 2010: **Drs. Lily and Yuh-Nung Jan**, University of California, San Francisco; 2009: **Dr. Jeremy Nathans**, Johns Hopkins University; 2008: **Dr. Michael Davis**, Emory University School of Medicine, Atlanta; 2007: **Dr. David Julius**, University of California, San Francisco; 2006: **Dr. Michael E. Greenberg**, Children's Hospital/Harvard Medical School; 2005: **Dr. Judith L. Rapoport**, National Institute of Mental Health/NIH; 2004: **Dr. Masakazu Konishi**, California Institute of Technology

The McGovern Institute for Brain Research at MIT conducts integrated research in neuroscience, molecular neurobiology, cognitive science, computation, and related areas.

Nominations

Please send nomination and supporting documents to:
Committee Chair, Edward M. Scolnick Prize in Neuroscience
McGovern Institute, MIT, 46-3160
77 Massachusetts Avenue
Cambridge, MA 02139
E-mail: mcgovern@mit.edu

For more information: <http://mcgovern.mit.edu>

POSITIONS OPEN

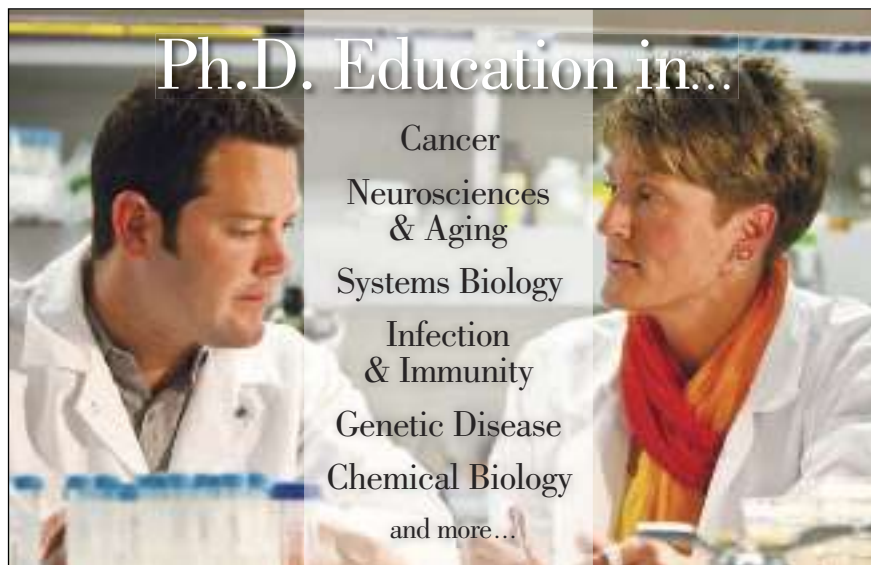


Metabolon is a diagnostics and services company offering the industry's leading biochemical profiling platform. We currently work with over 200 clients in the areas of pharmaceutical and biotechnology, nutrition, agriculture, bioprocessing and academic research. As part of Metabolon's expanding diagnostic and clinical testing business, Metabolon seeks an outstanding individual for the position of Senior Biostatistician. This person will be responsible for the identification of biomarkers from diagnostic metabolomic research studies, development of various statistical models and methods for analysis of metabolomic's data, and the development of any multivariate algorithms required for clinical tests. The ideal candidate will have experience with multivariate statistical modeling (genomics, proteomics or metabolomics data) and a proven track record of success as demonstrated from scientific peer-reviewed publications. The person selected for this position will work collaboratively with internal and external scientists on projects that lead to journal publications, presentations, and internal project progression reports. Ph.D. required. Interested applicants should send a letter of interest and CV to careers@metabolon.com.

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<http://www.metabolon.com>

CAREERS IN NEUROSCIENCE



Educating Ph.D. students to become the innovative biomedical scientists of the future.

Sanford-Burnham is a unique research environment that enjoys a culture of collaboration, a focus on new technologies and an emphasis on translating our basic research discoveries to improve human health.

Sanford|Burnham
Graduate School of Biomedical Sciences

<http://www.sanfordburnham.org/gradschool.aspx>

Sanford-Burnham Medical Research Institute is located on the Torrey Pines Mesa on the San Diego coast.

POSITIONS OPEN



Renal/Cardiac Research Faculty Positions

Two ASSISTANT/ASSOCIATE PROFESSOR level positions are open in the Hypertension and Vascular Research Division of Henry Ford Hospital to complement strengths in renal/cardiac cell and molecular biology and integrative physiology. Appointments will be in the Department of Internal Medicine at Henry Ford Hospital, Detroit, MI an affiliate of the Wayne State University School of Medicine with secondary appointments in basic science departments in the School of Medicine possible. The division's 8 basic scientists bring in more than \$6 million in grant support annually (<http://www.henryford.com/hypertensionresearch>). Successful applicants will develop and maintain robust, NIH-funded research programs. Minimum requirements are a Ph.D. or M.D., 3 years of postdoctoral experience, and a strong publication record. Salaries will be commensurate with experience. Positions come with generous startup packages and benefits.

To apply submit: a current CV; contact information for 4 references; and a 1-page description of research interests via e-mail in PDF format to: Emmett Bowen at ebowen1@hfhs.org. Positions will remain open until filled.

Henry Ford Hospital is an Equal Opportunity Employer.

CAREERS IN NEUROSCIENCE



STANFORD UNIVERSITY

Neuroscience Faculty Positions

The Stanford Institute for Neuro-Innovation and Translational Neurosciences (SINTN) is searching for three neuroscientist positions focused on a variety of areas, including the following:

- Investigation of the neural circuitry, molecular mechanisms and/or cellular physiology underlying movement disorders, including Parkinson's disease
- Neuroregeneration
- Neural mechanisms common to pain, addiction, or their intersection

The successful candidate(s) will be appointed at either the Assistant or Associate professor level in the University Tenure Line (UTL). The predominant criterion for appointment in the University Tenure Line is a major commitment to research and teaching.

Primary and secondary departmental affiliations are flexible and will be tailored for the successful candidate(s).

Please submit a curriculum vitae, statement of research interests and 3 reference letters by **December 15, 2010** to: Gary K. Steinberg, MD, PhD, Bernard and Ronni Lacroute-William Randolph Hearst, Professor of Neurosurgery and the Neurosciences, Director, SINTN, Chairman, Department of Neurosurgery, Stanford University School of Medicine, c/o Kristy Verhines, SINTN, 265 Campus Drive, G1078B, Stanford, CA 94305.

Please send an electronic copy of the cover letter and curriculum vitae to sintnjobs@stanford.edu.

Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women and members of minority groups, as well as others who would bring additional dimensions to the university's research and teaching missions.

Positions @ NIH

THE NATIONAL INSTITUTES OF HEALTH



Department of Health and Human Services
National Institutes of Health

Clinical Director
National Institute of Arthritis and Musculoskeletal and Skin Diseases

The Intramural Research Program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) is seeking a physician-scientist to serve as Clinical Director. This individual will direct the NIAMS Program in Translational Research, which includes training and clinical care branches as well as multiple investigative laboratories and branches. Investigators in the Clinical Program conduct studies in natural history and treatment as well as basic investigations into the etiology and/or pathophysiology of disease. The candidate should have the ability to manage this diverse clinical research enterprise and to provide the leadership to maintain the outstanding track record of the NIAMS Clinical Program. The ideal candidate for this position is an M.D. or M.D.-Ph.D. who is board-certified or board-eligible in either Pediatrics, Internal Medicine and Rheumatology or Allergy/Immunology. Potential areas of concentration would include rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, vasculitis, scleroderma, myositis, osteoarthritis, or other inflammatory/rheumatic diseases. The candidate should have experience in conducting clinical or translational research and in immunology, cell biology, genetics, or other areas of research relevant to rheumatic or autoimmune disease. The candidate will also be provided generous independent resources to develop his/her own clinical or translational research program as a tenured investigator within NIAMS.

The NIAMS Clinical Program is part of the NIH Intramural Research Program, located in Bethesda, Maryland. Investigators in this program have full privileges to admit their research subjects, free of charge to the patient, to the new Mark O. Hatfield Clinical Research Center, a state-of-the-art 230-bed hospital fully devoted to clinical and translational studies. Ample resources will be provided to the successful applicant to establish a first-rate Clinical Program and their own research program in his or her area of concentration. The NIAMS Intramural Research Program, headed by its Scientific Director, Dr. John O'Shea, comprises several outstanding programs in cytokine biology, signaling, genetics, and structural biology, and the broader NIH Bethesda campus provides a rich and highly interactive environment within a wide range of basic and translational research disciplines that relate directly to rheumatology.

Salary will be commensurate with experience. A full package of benefits, including retirement, health, life, and long-term care insurance, and a Thrift Savings Plan, is available. Qualified international applicants who have passed the ECFMG or USMLE examinations or have comparable qualifications are welcome to apply.

Interested applicants should send a curriculum vitae, a one-page summary of research interests, and the names of three referees to **Ms. Linda Peterson, 31 Center Drive, MSC 2350, Building 31, Room 4C12, Bethesda, MD 20892-2350, e-mail peterstonl@arb.niams.nih.gov**. If you need additional information, please call **Dr. John O'Shea at 301-496-2612**. Review of applications will begin on or about **January 3, 2011**, but applications will be accepted until the position is filled.



Help Us Help Millions

Division of Intramural Research Laboratory of Molecular Immunology

Postdoctoral positions are available in the Inflammation Biology Section of the Laboratory of Molecular Immunology, NIAID. The section uses the chemokine system to understand T-cell biology in humans and mouse models related to effector/memory cell function and differentiation, inflammation, host defense, and cancer. Principal, current projects focus on characterizing long-lived populations of memory cells in humans, understanding pathways for the differentiation of human CD4⁺ T cells, investigating the roles for chemokines/chemokine receptors in models of Th17-type inflammatory disease and host defense, understanding chemokine receptor signaling in lymphocytes, and using chemokine receptors as targets of novel probes for *in vivo* imaging. In consultation with the principal investigator, new members of the section will design independent projects related to these areas.

Application for these positions requires an interview. Contact Joshua M. Farber at jfarber@niaid.nih.gov. For more information about NIAID's available training and career opportunities, visit www.niaid.nih.gov/careers/DLIS.

National Institute of Allergy and Infectious Diseases

NIAID



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health



National Institute of Allergy and Infectious Diseases

Proud to be Equal Opportunity Employers



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Tenure/Tenure Track Investigator Position Laboratory of Immunology

The Laboratory of Immunology (LI), Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, invites applications for a tenure/tenure-track investigator position in immunology. Applicants should have a Ph.D., M.D., or equivalent degree; an outstanding record of postdoctoral accomplishment; and an interest in any area of biomedical research related to immunology.

Specifically, we seek a highly creative individual who will establish an independent, world-class research program that takes full advantage of the special opportunities afforded by the stable, long-term funding of the intramural research program at NIH. Applicants should be interested in developing and applying novel approaches to the study of problems of major biological and/or medical importance, which could include a significant clinical or translational effort in addition to bench research. The successful candidate would have access to the NIH Clinical Center, a state-of-the-art research hospital on the NIH campus in Bethesda, MD, and would have ample opportunity to participate in the activities of the Center for Human Immunology and other trans-NIH initiatives involving technology development, translational investigation, and multidisciplinary science.

Generous ongoing support for salary, technical personnel, postdoctoral fellows, equipment, and research supplies will be provided. Available cores or collaborative facilities include flow cytometry, advanced optical imaging, microarray generation and analysis, high throughput sequencing, computational biology, production of transgenic and gene-manipulated mice, biosafety level (BSL)-3 facilities, chemical genomics, and support for projects involving RNAi screening. In addition to an outstanding, international

postdoctoral community, a superior pool of graduate and undergraduate students is available to the successful applicant.

NIAID's Laboratory of Immunology has a distinguished history of accomplishment in immunology. We strongly encourage application by outstanding investigators who can continue and enhance this record of achievement. Current LI investigators are Ronald Germain, Michael Lenardo, David Margulies, Stefan Muljo, William Paul, Ethan Shevach, and Tsan Xiao.

To apply, e-mail your curriculum vitae, bibliography, and an outline of a proposed research program (no more than two pages) in PDF format to Ms. Bao-Hanh Ngo at LIT-TTSearch@niaid.nih.gov. In addition, three letters of reference must be sent directly from the referee to Drs. Giorgio Trinchieri and Dan Kastner, Co-Chairs, NIAID Search Committee, c/o Ms. Bao-Hanh Ngo at LIT-TTSearch@niaid.nih.gov or 10 Center Drive, MSC 1356, Building 10, Room 4A22, Bethesda, MD 20892-1356. E-mail is preferred.

Applications will be reviewed starting **December 13, 2010**, and will be accepted until the position is filled. For further information about this position, contact Dr. William Paul at 301-496-5046 or wpaul@niaid.nih.gov.

A full package of benefits (including retirement and health, life, and long-term care insurance) is available. Women and minorities are especially encouraged to apply. U.S. citizenship is not required.

Further information on working at NIAID is available on our Web site at www.niaid.nih.gov/careers/LIS.

National Institute of Allergy and Infectious Diseases



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health



National Institute of Allergy and Infectious Diseases

Proud to be Equal Opportunity Employers



Jagiellonian University
Max Planck Society



The Jagiellonian University in Krakow and the Max Planck Society announce a

Max Planck Research Group

in the area of

Molecular Plant Sciences
(including plant-microbe interactions)

The group will be located at the Jagiellonian University in Krakow.

The successful candidate should be early in her/his scientific career. The candidate is expected to develop an internationally competitive research program. Ideally, the scientific activities would complement ongoing research at the University and at the host institute of the Max Planck Society.

Funding covers a start-up equipment package (500,000 Polish Zlotys which is an equivalent to approx 150,000 Euro) and necessary and adequate running costs. In addition, funding is provided for the position of the group leader at the level of an associate professor (employed at a Max Planck Institute to be determined and detached to the Jagiellonian University in Krakow) and further staff members (one post-doctoral fellow post or two graduate students, one Ph.D. student and one technician) to be employed at the Jagiellonian University in Krakow. The initial appointment of the group leader is for 5 years with the possibility of extension (2 x 2 years) after international review.

Applications should include a CV, a list of publications, a one-page summary of scientific achievements, a two-page research plan and two letters of recommendation. **Deadline for application is December 16, 2010.** Successful applicants should be prepared to join a selection symposium held on **January 26+27, 2011 in Krakow, Poland.**

For further information and detailed application instructions see

<http://www.mprg.mpg.de>

The Max Planck Society in an equal opportunity employer, and is interested to raise the proportion of women in areas where they are underrepresented. Thus applications from female scientists are especially encouraged.



Oakland University
Department of Biological Sciences
Full Time Faculty Position
in Human Anatomy

The Department of Biological Sciences at Oakland University invites applicants for a full-time faculty position in human anatomy to be filled by August 2011. The appointment will be for a Special Instructor, which is a teaching-intensive faculty position leading to job security equivalent to tenure. The Department offers laboratory courses in human anatomy utilizing cadaver dissection and dissections as well as human anatomy lecture courses to a population of mainly preprofessional and health sciences students. An optimal candidate will have a Ph.D. in anatomy, but applicants with other relevant doctoral degrees or a master's degree plus experience will also be considered. This individual will have excellent teaching skills and will be able to teach human anatomy effectively at the undergraduate level, mentor teaching assistants in the human anatomy labs, and work collaboratively with other participating faculty and programs. The position offers a competitive salary package and outstanding retirement and health benefits.

The Department of Biological Sciences (<http://www2.oakland.edu/biology/>) is a modern, well equipped, research oriented department with state of the art instructional labs including a dedicated cadaver lab. Oakland University is a state-supported institution of over 19,000 students situated on a beautiful 1,500-acre wooded campus in the northern metropolitan Detroit area.

Applicants should mail their curriculum vitae including a list of references and teaching philosophy and arrange to have three letters of recommendation sent to: **Human Anatomy Search Chair, Department of Biological Sciences, Oakland University, Rochester, MI 48309-4401.** Review of applications will begin January 15, 2011 and continue until the position is filled. Questions can be addressed to wendell@oakland.edu.

Oakland University is an equal opportunity employer.



www.westernu.edu

Faculty Positions in Microbiology/Immunology available for 2011

College of Osteopathic Medicine of the Pacific – Northwest, Lebanon, Oregon

Western University of Health Sciences, a thriving center for human health care and veterinary medicine education, is opening a new site for the College of Osteopathic Medicine of the Pacific – Northwest (COMP-NW) in Lebanon, Oregon with the inaugural class beginning in Fall, 2011. The University's 10 year plan and core values have propelled the Institution to be a benchmark University for the development of interprofessional and graduate medical education. The University values a diverse institutional community and is committed to excellence in its faculty, staff and students. Western University seeks applicants of distinguished academic accomplishments who possess a passion for excellence and can illustrate a proven track record of achievements.

The Department of Basic Medical Sciences provides the preclinical education for the College of Osteopathic Medicine, and invites applications from highly motivated individuals for tenure-track faculty positions in microbiology/immunology. These are full-time, 12-month, tenure-track positions at the Assistant Professor/Associate Professor/Professor rank dependent upon qualifications. Successful candidates will be located at the new site on the COMP-NW campus. Applicants must have a Ph.D. in microbiology or immunology and at least 2 years of postdoctoral experience. Similar positions in physiology, biochemistry/genetics, and pharmacology are also available at both the Lebanon, OR and Pomona, CA campuses. Preference will be given to master educators who have demonstrated excellence in teaching with significant scholarly activity and/or those with a history of extramural funding and a strong potential to obtain further grant support for their research program. Submit a current curriculum vitae and a cover letter describing your teaching experience and philosophy, your research activity and goals, and how you meet the qualifications for the position. Please include contact information for at least three references. These positions will remain open until filled.

Nissar A. Darmani, PhD

Assistant Dean for Basic Sciences and Research
Department of Basic Medical Sciences
College of Osteopathic Medicine of the Pacific
Western University of Health Sciences
309 E. Second Street, Pomona, CA 91766-1854
Email Address: ndarmani@westernu.edu

Western University of Health Sciences is an equal opportunity employer.



UNIVERSITY of OULU
OULUN YLIOPISTO

*The University of Oulu in Northern Finland is an international, multidisciplinary research university with a rich pool of creative and intellectual talent. The Oulu Region is recognized as a world-class R&D hub with R&D input per capita among the highest globally. We promote advanced research in six faculties and more than 70 different specialist disciplines especially in our focus areas: **Biosciences and Health – Environment, Natural Resources and Materials – Information Technology – Cultural Identity and Interaction.***

We are now offering outstanding researchers with PhD's **Investigator Start-up Packages** for five years

Research Fellow start-up packages in

1. Biosciences and Molecular Medicine in Biocenter Oulu (2 positions)
2. Experimental NMR Spectroscopy
3. Mathematical Analysis
4. Biomedical Engineering with focus on Multimodal Characterization and Modelling of Tissues and Organs
5. River Basin Research
6. Interdisciplinary and Transdisciplinary Research in Environmental Sciences
7. Sustainable Resource Management and Material Efficiency
8. Communication, Sensing and Networking Challenges of Nano-scale Wireless Sensor Networks

9. Human Geography with a research theme Mobilities, borders and identity
10. The Northern Finland Birth Cohorts 1966 and 1986 Study

Professorship in

11. Education with a special emphasis on Learning and Interaction in Teacher Education

In addition, we are looking for outstanding Post- Doctoral Researchers in

12. Economic Geology and Petrology with a focus on the Petrogenesis of Platinum Deposits
13. Materials Research Using Synchrotron Radiation Excited Electron Spectroscopy
14. Genes and Environment (2 positions)

More information on the positions and how to apply at www.oulu.fi/english





CIFAR's Junior Fellow Academy Announces New Fellowships

The Junior Fellow Academy of the Canadian Institute for Advanced Research is an elite fellowship program designed to build research and leadership capacity in gifted scholars at a critical early stage of career development. The Academy provides unique opportunities for personal and professional growth through close collaboration with and mentorship from some of the best researchers in Canada and around the world.

By participating both in an innovative CIFAR research program and the leadership-building Junior Fellow Academy, Junior Fellows learn to embrace CIFAR's core values: to think broadly and imaginatively across disciplines and to collaborate on a deep level with colleagues. These valuable experiences are intended to profoundly impact a Junior Fellow's future career path.

CIFAR Junior Fellowships are held in conjunction with a university appointment. Most typically, Junior Fellows work as postdoctoral fellows under the direct supervision of one or more CIFAR program members. Junior Fellow applicants in the social sciences and humanities may hold a junior faculty position.

Eligibility: The Academy is targeted to individuals who have completed or will complete their PhD within the three years prior to advertised Junior Fellowship starting dates and have demonstrated outstanding scholarship and research potential. Advertisements for Junior Fellowship positions currently available in various CIFAR research programs may specify additional eligibility requirements.

Duration: Two years.

Value*: **For postdoctoral fellows (per year):**

- \$70,000 CDN/US for salary and benefits
- \$5,000 CDN/US for research support

For junior faculty members (per year):

- \$50,000 CDN/US for research support

**US dollar values apply when positions are held outside of Canada.*

How to Apply: Specific Junior Fellowship positions in several CIFAR programs, as well as application instructions, are now advertised at www.cifar.ca/JFA, with an **application deadline of January 21, 2011**. New positions and deadlines will be posted each fall and spring. Visit today for more information about CIFAR and its Junior Fellow Academy.

CIFAR is strongly committed to diversity within its community, and especially welcomes applications from visible minority group members, women, Aboriginal persons, persons with disabilities, members of sexual minority groups and others who may contribute to further diversification of ideas.

CIFAR's Research Programs:

- **Cosmology and Gravity;** J. Richard Bond, Director
- **Earth System Evolution;** Jerry Mitrovica, Director
- **Experience-based Brain and Biological Development;** Tom Boyce and Marla Sokolowski, Directors
- **Genetic Networks;** Brenda Andrews, Director
- **Institutions, Organizations and Growth;** Elhanan Helpman, Director
- **Integrated Microbial Biodiversity;** Patrick Keeling, Director
- **Nanoelectronics;** Peter Grütter, Director
- **Neural Computation and Adaptive Perception;** Geoffrey Hinton, Director
- **Quantum Information Processing;** Raymond Laflamme, Director
- **Quantum Materials;** Louis Taillefer, Director
- **Social Interactions, Identity and Well-Being;** George Akerlof and John Helliwell, Directors
- **Successful Societies;** Peter A. Hall and Michèle Lamont, Directors



Tenure-Track Position Graduate School of Nanoscience and Technology KAIST (Korea Advanced Institute of Science and Technology)

The Graduate School of Nanoscience and Technology at KAIST invites applications for full-time, tenure-track or tenured faculty positions.

The Graduate School of Nanoscience and Technology is a recently established department in the College of Natural Sciences at KAIST, specializing in the interdisciplinary research in the area where physics, chemistry, biology, materials science, and engineering come together. In particular, we are interested in the following three areas of nanoscience research: Bio-photonics, including imaging, sensing, and manipulation; Fabrication/synthesis/characterization of nanomaterials and structures; and theoretical/computational nanoscience research. However, qualified applications will be given equal consideration regardless of the proposed field of research.

Applicants must have a Ph.D., and some postdoctoral research experience is desirable. However, candidates from all fields, nationality, and ethnicity will be considered equally, and are encouraged to apply. The position is for a junior level professor, but senior level appointments are possible depending on qualifications.

Interested candidate should submit official application form (available at http://www.kaist.edu/english/02_faculty/01_recruit_02.php?pt=22) including publication list, research plan and teaching plan and at least three letters of recommendation to **Prof. Jung H. Shin, Head, Graduate School of Nanoscience and Technology, KAIST, Daejeon, 305-701, Korea.**

Applications completed by December 24, 2010, will be given full consideration, but the search will continue until the positions are filled.

If you have any further inquiry, please contact the Program Head at jhs@kaist.ac.kr.



FACULTY POSITION

The Department of Virology and Immunology at the Southwest Foundation for Biomedical Research in San Antonio, Texas, invites applications for a faculty-level position for the first Ewing Halsell Scholar. The appointment will be at the SCIENTIST level, this is equivalent to a full professor at a major research university. The Department has faculty with research programs focusing on hepatitis viruses, human and simian immunodeficiency viruses, and emerging viruses such as hemorrhagic fever viruses. Major strengths of the Foundation are the extensive nonhuman primate resources, which include macaques, marmosets, chimpanzees and baboons.

There is a strong pre and postdoctoral training program and a close association with the University of Texas Health Science Center at San Antonio, including a role in graduate education. All candidates must have as a minimum a doctoral degree in the biological sciences or an M.D. degree, and have completed at least two years of relevant postdoctoral research. Candidates must have demonstrated ability to direct an independent research program in animal virology and have been shown to be competitive for extramural support. Funding for this position comes from the Ewing Halsell Foundation.

All areas of virology will be considered but preferences will be given to emerging viral agents that require maximum containment and the use of nonhuman primates. The Department is particularly interested in a person with expertise in host-pathogen interactions with an innovative program in molecular pathogenesis or cellular immunology.

Please mail curriculum vitae, a description of research interest, and the contact details for three references to: **Director of Human Resources, Southwest Foundation for Biomedical Research, P.O. Box 760549, San Antonio, Texas 78245-0549; www.SFBR.org.**

EOE

SUSTAINABLE ENERGY FOR A BETTER WORLD

The **Master of Engineering (Sustainable Energy)** program at RMIT University, Melbourne, Australia, offers engineering and science graduates a unique opportunity to gain a specialist qualification in the rapidly growing area of sustainable energy.

The program has a strong applied focus on the full range of energy efficiency and renewable energy technologies including solar thermal, biomass, wind and wave power, and cogeneration and trigeneration.

A unique feature of the program is an innovative approach to setting emerging technologies and technical analysis firmly within the context of economic, environmental, and social evaluation of sustainability. Hence RMIT Masters graduates are uniquely equipped to become effective sustainable energy practitioners in the global industry, governmental, nongovernmental and community-based organisations.

Specialists teaching the program include international experts and award winning Australian researchers.

The program has attracted highly talented students from around Australia and the world who are committed to creating a better world.

**For further information contact, Associate Professor John Andrews
School of Aerospace, Mechanical and Manufacturing Engineering
Email john.andrews@rmit.edu.au or phone 61 3 9925 6085**

www.rmit.edu.au/aeromecheng/sustainableenergy



S1624

GRADUATE PROGRAM

CALL FOR PhD STUDENTS

The Graduate School at IST Austria invites applicants from all countries to its PhD program. IST Austria is a new institution located on the outskirts of Vienna dedicated to cutting-edge basic research in the natural sciences and related disciplines. The language at the Institute and the Graduate School is English.

The PhD program combines advanced coursework and research, with a focus on Biology, Computer Science, Neuroscience, and interdisciplinary areas. IST Austria offers internationally competitive PhD salaries supporting 4-5 years of study. Applicants must hold either a BS or MS degree or equivalent.

The Institute offers PhD students positions with the following faculty:

- **Nick Barton** Evolutionary and Mathematical Biology
- **Jonathan P. Bollback** Evolutionary Biology
- **Tobias Bollenbach** Biophysics and Systems Biology
- **Krishnendu Chatterjee** Game Theory and Software Systems Theory
- **Sylvia Cremer** Evolutionary and Behavioral Biology
- **Herbert Edelsbrunner** Algorithms, Geometry, and Topology
- **Călin C. Guet** Systems and Synthetic Biology
- **Carl-Philipp Heisenberg** Cell and Developmental Biology
- **Thomas A. Henzinger** Software Systems Theory
- **Peter Jonas** Neuroscience
- **Christoph Lampert** Computer Vision and Machine Learning
- **Michael Sixt** Cell Biology and Immunology
- **Gašper Tkačik** Theoretical Biophysics and Neuroscience
- **Chris Wojtan** Computer Graphics

Additional faculty members will be announced on the IST website www.ist.ac.at.

**CAMPUS
VISIT DAY**
November 27,
2010

For further information and access to the online application please consult www.ist.ac.at/gradschool. For inquiries, please contact gradschool@ist.ac.at. For students wishing to enter the program in the fall of 2011, the deadline for applications is **January 15, 2011**.

IST Austria is committed to Equality and Diversity. Female students are encouraged to apply.



Faculty Position - Computational Biology

The Computational Biology Program and the Division of Public Health Sciences of the Fred Hutchinson Cancer Research Center invites applications for the position of Assistant or Associate Faculty Member (equivalent, respectively, to Assistant or Associate Professor at a university). This position will include a primary appointment in the Computational Biology Program and in the Division of Public Health Sciences, and possibly an appointment in Basic Sciences, Human Biology, Vaccine and Infectious Disease, or Clinical Divisions, depending on mutual interest.

The successful candidate will enter an active and dynamic research environment with many opportunities to collaborate with scientists across a diverse range of disciplines. We are seeking outstanding candidates with a strong research program that includes computational strategies, possibly in combination with their own laboratory investigation, to investigate important biological phenomena. All areas will be considered, but we are particularly interested those relating to: genomics, infectious diseases, cancer biology, basic biology, and population sciences. The incumbent will be expected to conduct an active program of independent and collaborative research pertinent to the mission of the Center. For more information on the Computational Biology Program, please go to: <http://compbio.fhcr.org/>.

An affiliate appointment at the Assistant or Associate professor level in a relevant department at the University of Washington is possible, depending on mutual interest. Applicants should have a PhD or equivalent degree. Faculty rank will be dependent on qualifications and experience. The Fred Hutchinson Cancer Research Center is a world-renowned research institution. The Center conducts research of the highest standards to improve prevention and treatment of cancer and other diseases related to human health.

The applicant review process begins as applications arrive, but any application received by **December 1, 2010**, will be guaranteed consideration for the position. Applications that arrive after that date will be considered as long as the position remains open. Please send applications including curriculum vitae, a letter describing research interests, and the names of four references by e-mail or mail to:

Martin McIntosh, Search Committee Chair
c/o Sara Zriny
Division of Public Health Sciences M1-C108
Fred Hutchinson Cancer Research Center
1100 Fairview Avenue North, PO Box 19024
Seattle, Washington 98109-1024
email: Sara Zriny (szzriny@fhcr.org)

*The Fred Hutchinson Cancer Research Center and the University of Washington are Equal Opportunity Employers.
Both institutions are building culturally diverse faculty and strongly encourage applications from female and minority candidates.*



Oakland University Department of Biological Sciences Tenure-Track Assistant Professor Position in Human Physiology

The Department of Biological Sciences at Oakland University invites applications for a tenure-track Assistant Professor position to be filled by August 2011. We seek a candidate with a strong background in physiology or in physiology with pharmacology who will investigate key problems in cellular or systemic physiology. Laboratory space and a competitive startup package will be provided. A PhD and post-doctoral experience are required as well as a strong research track record evidenced by publications and grant activity. The successful candidate is expected to develop a vigorous, extramurally funded research program, to teach effectively at the undergraduate and graduate levels, and to mentor undergraduate and graduate students (MS and PhD).

The Department of Biological Sciences (<http://www2.oakland.edu/biology/>) is a modern, well equipped, and research oriented department of 22 faculty members. Oakland University is a state-supported institution of 19,000 students situated on a beautiful 1,600-acre campus 25 miles north of Detroit.

Applicants should mail a hard copy of application including their curriculum vita, statements of research plans and teaching philosophy, copies of key reprints, and arrange to have three letters sent by January 7, 2011 to: **Anne Hitt, Physiology Search Chair, Department of Biological Sciences, Oakland University, Rochester, MI 48309-4401**. Please communicate any inquiries by email to hitt@oakland.edu or dvir@oakland.edu.

Oakland University is an Affirmative Action/Equal Opportunity Employer and encourages applications from women and minorities.



TEXAS TECH UNIVERSITY

Edward. E. Whitacre Jr.
College of Engineering

The Maddox Chairs in Energy at Texas Tech University

The Edward E. Whitacre Jr. College of Engineering at Texas Tech University is committed to leveraging these **two exceptionally large endowed chairs at over \$7 million each**, to become one of the nation's leaders in finding solutions to the world's energy challenges. The college is seeking world-class researchers in solar and sustainable energy as candidates for the Maddox Chairs.

Donovan Maddox Distinguished Engineering Chair in Solar Energy Jack Maddox Distinguished Engineering Chair in Sustainable Energy

Candidates are expected to have an international reputation in the fields of solar energy or energy sciences and engineering as evidenced by publications, citations, and peer recognition. In addition, a record of acquiring external resources to support research, team building, and mentoring of associates and graduate and undergraduate students is necessary.

The successful candidates will set the tone, vision, and path in order to build an internationally recognized program at Texas Tech University in solar and sustainable energy research. The appointments will be as a full professor in the Whitacre College of Engineering.

The holders of each of the Maddox Chairs will be expected to not only bring his or her own research activities to the Whitacre College of Engineering, but also to build a collaborative community of scholars at Texas Tech to build a world-class research program.

Screening will begin upon the receipt of applications and will continue until the position is filled. Candidate names will not be made public until the final stages of the search. Curriculum vitae and the names and contact information of at least four references should be submitted at www.coe.ttu.edu/maddox. To nominate a colleague for these chairs, visit www.coe.ttu.edu/maddox. Nominations can be made anonymously.

Questions about the Jack Maddox or Donovan Maddox Chairs should be directed to:
Jack Maddox and Donovan Maddox Search Committees
Texas Tech University | Whitacre College of Engineering
Box 43103 | Lubbock, Texas 79409-3013 | engineeringdean.coe@ttu.edu | 1.800.528.5583

Texas Tech University | Whitacre College of Engineering
1.800.528.5583 | www.coe.ttu.edu/maddox

An Affirmative Action/Equal Opportunity
Institution with Diverse Faculty



KYUSHU UNIVERSITY

International Institute for Carbon-Neutral Energy Research

The International Institute for Carbon-Neutral Energy Research (I²CNER) at Kyushu University in Japan is actively seeking candidates for open-rank faculty and post-doctoral research associates.

Outline: The International Institute for Carbon-Neutral Energy Research (I²CNER) is a member of the World Premier International Research Center Initiative established by the Japanese Ministry of Education, Culture, Science and Technology (MEXT). Faculty members and researchers associated with I²CNER are dedicated to the Institute's mission to contribute to the creation of a sustainable and environmentally friendly society by advancing fundamental science to reduce CO₂ emissions and the realization of a hydrogen economy. The ITO Campus of the Kyushu University houses extensive state-of-the-art experimental and computational facilities.

Qualifications: The successful candidate will have experimental or computational expertise in the physics, chemistry, mechanics, and materials science aspects of:

- Hydrogen storage and embrittlement
- Next generation fuel cells for transportation
- Solar/chemical hydrogen production
- Thermophysical properties of hydrogen and CO₂
- Chemistry for efficient material transformation
- Basic science issues underlying CO₂ separation/concentration and geologic/oceanic capture and sequestration

Faculty members are expected to initiate and sustain vigorous research programs that both advance and are relevant to the mission of I²CNER. Candidates for faculty positions must have achieved national and international recognition for their research accomplishments. Post-doctoral research associates will join research groups relevant to their field of expertise and education.

Application process: Applicants must provide (1) cover letter; (2) curriculum vitae that details research experience and interests; (3) a list of publications; (4) the names and contact information of four references. All materials must be submitted in English.

Starting Date: As soon as possible after closing date

Salary: Commensurate with qualifications and experience

Application deadline: 17:00, December 24, 2010 (Japan Time); Interviews may take place prior to the closing date; however, no final decisions will be made until after this time.

For More Information: <http://www.kyushu-u.ac.jp/english/research/wpi/>;

Send inquiries to: wpi-office@i2cner.kyushu-u.ac.jp

Kyushu University is an Equal Opportunity/Affirmative Action Employer. The administration, faculty and staff embrace diversity and are committed to attracting qualified candidates who also embrace and value diversity and inclusivity.

The Lundbeck Foundation Junior Group Leader Fellowships

The Lundbeck Foundation hereby invites applications for five fellowships which will be granted to especially promising young researchers and their research groups.

The fellowships are awarded for five years, and each fellowship amounts to DKK 10 mio. (approx. Euro 1,3 mio.).

The fellowships are intended for five researchers in their thirties who are qualified to establish or develop their own research groups within the health- or natural sciences. We envisage researchers who have received their Ph.D. degree within the last 5-7 years. The subject area should be frontline basic- or applied research within the scope of the Foundation's grant strategy, as can be seen at www.lundbeckfonden.dk

The fellowships may well attract Danish or foreign researchers from abroad who wish to move to Denmark and continue their research here. The call is also open for researchers from Danish universities and university hospitals.

The application should include an account of the project's research plan, collaborators, budget and how the research group is envisioned to be placed within a Danish research institution. In addition, a curriculum vitae, a list of scientific publications and letters of recommendation are requested.

The application, written in English, should be sent via the Foundation's Electronic Application System for Junior Group Leader Fellowships 2011 at www.lundbeckfonden.dk no later than December 15, 2010.

For further information please contact Anne-Marie Engel, Director of Research at the Lundbeck Foundation, phone: (+45) 39 12 80 17 or at mail@lundbeckfonden.dk

The Lundbeck Foundation is a commercial foundation with considerable shareholdings in the two listed companies H. Lundbeck A/S and ALK-Abelló A/S. Yields from the Foundation's capital are used, among other things, to support scientific research primarily within the biomedical sciences but also the biologically oriented natural sciences as well as physics and chemistry. The Foundation grants approx. DKK 400 mio. (approx. Euro 50 mio.) annually to scientific research.

Lundbeckfonden
Vestagervej 17, DK-2900 Hellerup
Tel. +45 39 12 80 00
www.lundbeckfonden.dk

LUNDBECKFONDEN



UCIrvine

THE HENRY SAMUELI
SCHOOL OF ENGINEERING

TENURE-TRACK AND TENURED FACULTY POSITIONS

THE HENRY SAMUELI SCHOOL OF ENGINEERING AT THE UNIVERSITY OF CALIFORNIA, IRVINE invites qualified applicants for a tenure-track faculty position. The position will be formally associated with Biomedical Engineering Department and will interact closely with the Laboratory for Fluorescence Dynamics, the Beckman Laser Institute, the Edwards Lifesciences Center for Advanced Cardiovascular Technologies and the Micro/nano Fluidics Fundamentals Focus Center. Applicants must hold a Ph.D. degree in biomedical engineering or related field, and will be expected to maintain a broad-based extramurally funded research program. Research areas of interest include broadly the application of engineering technologies (e.g. bionanotechnology) to address the development and application of synthesized biomolecular probes to dynamically probe cellular and molecular events, both in vivo and in vitro. The biomolecular probes can be broadly defined including, but not limited to, nanoparticles (either organic or inorganic), aptamers, FRET and SERS based probes, quantum dots, etc.. Applications for these biomolecular probes are often referred to as "nanomedicine" which include, but are not limited to, diagnosing and treating cancer, heart/brain diseases, and for studying basic biological processes (e.g. live cell imaging, mechanobiology, real-time monitoring and quantifying of genes, contrast agents for in vivo imaging, and delivery of therapeutic molecular reagents in vivo). In addition, the successful candidate will be expected to advise students and teach undergraduate and graduate courses as well as develop collaborative programs with other faculty members and programs at UCI. The University of California, Irvine is situated in Orange County's rapidly growing high technology sector that includes more than 310 biomedical companies which are actively involved in our program. Nominally, the position will be at the assistant professor level (tenure-track), but in exceptional cases senior applicants will be considered. The successful candidate will be considered for a Samueli Faculty Career Development Professorship (junior level) or a Samueli Endowed Chair (senior level).

APPLY NOW – submit your application to our online recruitment program.

For full consideration, candidates should upload applications electronically, please refer to the following website for instructions: <http://www.eng.uci.edu/employment/applicationinstructions>. Applications should include a curriculum vitae, a brief (no more than 2 pages) description of current and future research and teaching interests, and names of at least three references. Questions regarding these positions may be addressed to Ms. Ruth M. Gratzter, rmgratzte@uci.edu. For more information about the Department of Biomedical Engineering please visit our website at <http://www.bme.uci.edu>. Selection will begin December 1, 2010, and continue until the position is filled.

UCI is an Equal Opportunity Employer committed to excellence through diversity and strongly encourages applications from all qualified applicants, including women and minorities. UCI is responsive to the needs of dual career couples, is dedicated to work-life balance through an array of family-friendly policies, and is the recipient of an NSF ADVANCE Award for gender equity.

Faculty Position in Canine Genetics/Genomics

The Cornell University College of Veterinary Medicine invites applications for an open rank tenure-track faculty position in statistical genetics, computational genomics, or complex trait genetics. The successful individual is expected to develop and lead a strong research program in canine genetics/genomics, preferably focusing on the genetics of disease. The individual will engage with clinical and basic researchers at the College – considered to be the world's best – and across the University. He/she may participate in the Center for Comparative and Population Genomics, Baker Institute for Animal Health, Center for Vertebrate Genomics, and canine DNA Bank. An established individual would be expected to play a leadership role in Cornell's program in Comparative Canine Genomics. Cornell has excellent graduate programs in Statistical Genomics, Computational Biology and Statistics, Biomedical Sciences, and Molecular Genetics. The University has state-of-the-art genomics core facilities.

Cornell's main campus is situated in the beautiful Finger Lakes region of upstate NY. Cornell embraces diversity and seeks candidates who will create a climate that attracts student of all races, nationalities and genders. Women and underrepresented minorities are strongly encouraged to apply. Cornell also seeks to meet the needs of dual career couples.

To apply, please submit a curriculum vitae and a description of current and future research interests, with an emphasis on canine research, as a single PDF file to Dr. John Schimenti (doggenetics@cornell.edu). Also, arrange for three letters of recommendation to be sent electronically to the above address. The committee will begin considering applications December 1, 2010.



Cornell University

*Cornell University is an Affirmative Action/
Equal Opportunity Employer and Educator.*

UNIVERSITY OF CALIFORNIA
UCRIVERSIDE

FACULTY POSITION in Biophysics/Structural Biology Department of Biochemistry

As part of an on-going programmatic initiative, the Department of Biochemistry at the University of California, Riverside invites applications for a new faculty position at the Assistant Professor level in the general area of biophysics, specifically including (but not limited to) structural biology (e.g., X-ray crystallography and biomolecular NMR). The successful candidate will be expected to develop a vigorous, independent, and innovative research program that is able to attract extramural funding, and also to contribute to the Department's undergraduate and graduate teaching missions. The candidate will also be expected to interact with existing faculty on campus in the development of a vigorous graduate program in biophysics. The position is part of a continuing effort at UCR to expand structural biology and biophysics on campus, particularly in areas of biomedical relevance, and build on recent hires in these areas. A competitive startup package will be provided, with salary commensurate with education and experience. The position is available July 1, 2011. A Ph.D., M.D., or equivalent degree is required.

Interested individuals should send *curriculum vitae*, a brief statement of research interests, and arrange for at least three letters of reference to be sent to **Chair, Biophysics and Structural Biology Search Committee, Department of Biochemistry, University of California, Riverside, CA 92521-0129**. The University of California, Riverside is recognized as one of the Nation's most ethnically and culturally diverse campuses, and applications from highly qualified women and underrepresented minorities are particularly encouraged. Electronic applications are encouraged (send to Biochemistryjobs@ucr.edu). Review of applications will begin **November 30, 2010**, and will continue until the position is filled. For additional information about the Department, visit (<http://biochemistry.ucr.edu>), and for the campus, visit <http://www.ucr.edu>.

*The University of California is an
Affirmative Action/Equal Opportunity Employer.*

In 2011,
CNRS
is recruiting
377 permanent
researchers
in all scientific fields

- LIFE SCIENCES
- CHEMISTRY
- ENVIRONMENTAL SCIENCES
AND SUSTAINABLE
DEVELOPMENT
- HUMANITIES AND SOCIAL
SCIENCES
- COMPUTER SCIENCE
- ENGINEERING
- MATHEMATICS
- PHYSICS
- NUCLEAR AND PARTICLE
PHYSICS
- EARTH SCIENCES AND
ASTRONOMY

Online registration at www.cnrs.fr
from December 1, 2010
Registration deadline
January 6, 2011

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Help give science a greater voice in Washington, DC! Since 1973, AAAS Fellows have applied their skills to federal decision-making processes that affect people in the U.S. and around the world, while learning first-hand about the government and policymaking.

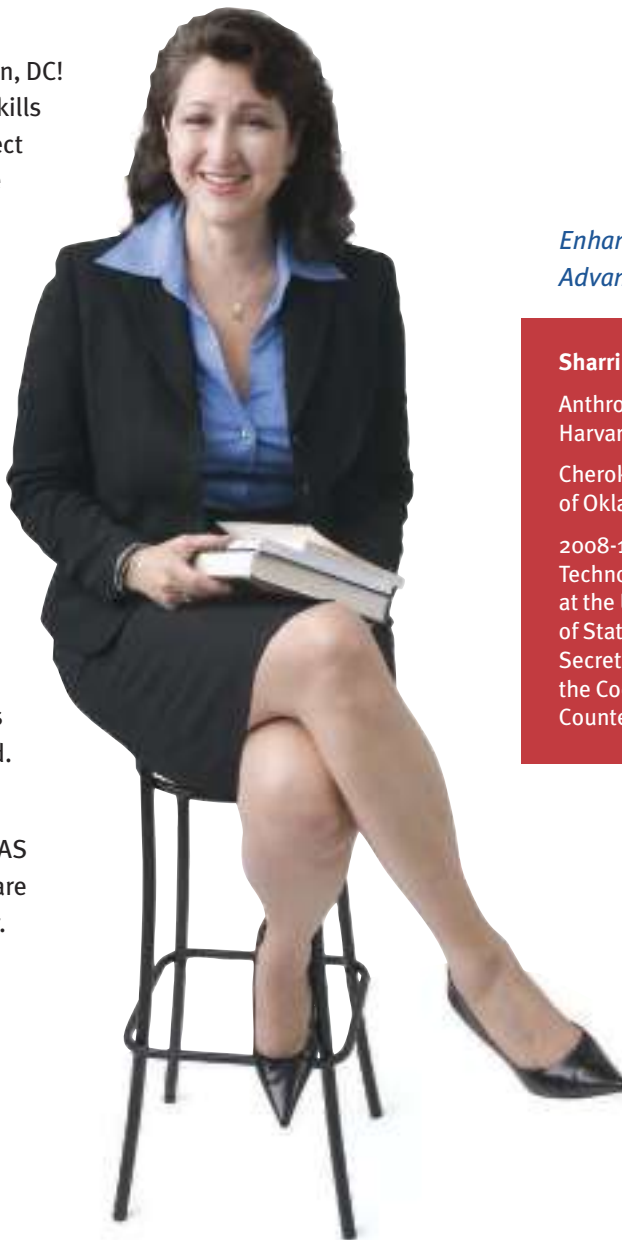
Join the Network.

Year-long fellowships are available in the U.S. Congress and federal agencies. Applicants must hold a PhD or equivalent doctoral-level degree in any behavioral/ social, biological, computational/ mathematical, earth, medical/health, or physical science, or any engineering discipline. Individuals with a master's degree in engineering and three years of post-degree professional experience also may apply. Federal employees are not eligible and U.S. citizenship is required.

Apply.

The application deadline for the 2011-2012 AAAS Fellowships is 5 December 2010. Fellowships are awarded in the spring and begin in September. Stipends range from \$74,000 to \$97,000.

Note: Additional fellowships are available through approximately 30 scientific society partners. Individuals are encouraged to apply with AAAS as well as with any scientific societies for which they qualify.



*Enhancing Public Policy,
Advancing Science Careers*

Sharri Clark, PhD

Anthropology,
Harvard University

Cherokee (Cherokee Nation
of Oklahoma) and Choctaw

2008-10 AAAS Science &
Technology Policy Fellow
at the U.S. Department
of State, Office of the
Secretary, Office of
the Coordinator for
Counterterrorism



Full details at: **fellowships.aaas.org**



University of
Massachusetts
UMASS Medical School

Diabetes
CENTER OF EXCELLENCE

FACULTY POSITION

The Diabetes Center of Excellence at the University of Massachusetts Medical School invites applications for a **SENIOR TENURED** or **JUNIOR TENURE-TRACK** basic science or clinical investigator faculty position. The Diabetes Center of Excellence currently consists of basic and physician scientists representing a broad range of disciplines in the biomedical and clinical sciences, with members from several Medical School Departments and Programs working together as central New England's leading center for diabetes clinical care, innovation, and discovery. The Center occupies state-of-the-art research space on an expanding Medical School campus, and benefits from being a continuously funded Diabetes-Endocrinology Research Center (DERC) for over 28 years. Basic investigators benefit from core facilities for deep sequencing, proteomics, genotyping, fluorescence-activated cell sorting, digital imaging/confocal microscopy, genomics/bioinformatics, transgenic/knockout mice, and mouse metabolic phenotyping. Clinical and community investigators benefit from infrastructure for recruitment and retention of research participants, measurement, behavioral and nutritional intervention resources and facilities, and academic-community research partnerships. Adult and pediatric clinical efforts of the Diabetes Center are housed in a new Ambulatory Clinical Care building designed so that patients can receive appropriate retinal photos, foot care, diabetes education, and other routine medical care at one location. The position will be highly competitive with regard to start-up funds, laboratory space and salary. The Center seeks an individual of outstanding research potential relating to diabetes pathophysiology or treatment.

Applicants should send curriculum vitae, statement of research interests, and names and addresses of three references to:

Dr. David M. Harlan
Co-Director, Diabetes
Center of Excellence
Search Committee Chair
% Patricia Cannon
55 Lake Avenue North
Ambulatory Care Building,
Room AC4.127
Worcester, MA 01655
Patricia.cannon@umassmed.edu
508.856.3800

As an Equal Opportunity and Affirmative Action Employer, UMMS recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives, and backgrounds.



Computational Biology, University of Pennsylvania School of Medicine

The Center for Bioinformatics at the University of Pennsylvania's School of Medicine seeks candidates for several Full, Assistant, and/or Associate Professor positions in the tenure track. Rank will be commensurate with experience. The successful applicant will have experience in the field of bioinformatics. Responsibilities include research and teaching in bioinformatics and computational biology, and participation in the Genomics and Computational Biology graduate program. Applicants must have an M.D. and/or Ph.D degree and have demonstrated excellent qualifications in Research.

This recruitment is directed at individuals who specialize in bioinformatics. The search represents the combined effort of the Penn Center for Bioinformatics and the Institute for Translational Medicine and Therapeutics. Each faculty appointment will be in a School of Medicine department that is consistent with the scientific expertise of each successful candidate.

Departments for faculty appointment include:

Genetics: Seeking candidates in computational genomics, statistical genetics, population or evolutionary genetics, analysis of complex genetic diseases, and network biology.

Microbiology: Seeking candidates who study host-pathogen interactions utilizing large data-sets generated by deep-sequencing, whole genome RNAi screening, microarrays and multiparametric FACS assays.

Pharmacology: Seeking candidates in translational informatics, pharmacogenomics, network biology, bioinformatics, and associated disciplines.

Biostatistics and Epidemiology: Seeking candidates that develop novel statistical, probabilistic, and computational methods for analysis of large-scale genetic and genomic data.

Medicine: Seeking physician-scientist candidates who use computational approaches to medical genetics and/or translational medicine.

Pathology and Laboratory Medicine: Seeking candidates in the areas of computational medicine including image analysis and high throughput molecular data that are adaptable to the creation of new diagnostic modalities.

Apply for this position online at: http://www.med.upenn.edu/apps/faculty_ad/index.php/g/d2447

The University of Pennsylvania is an Equal Opportunity, Affirmative Action Employer. Women and minority candidates are strongly encouraged to apply.

WOODS HOLE OCEANOGRAPHIC INSTITUTION

Fellowships for Postdoctoral Scholars

New or recent doctoral recipients with research interests associated with the following are encouraged to submit scholarship applications prior to Jan. 15, 2011.

DEPARTMENTS - Awards related to the following areas are anticipated: Applied Ocean Physics & Engineering; Biology; Geology & Geophysics; Marine Chemistry & Geochemistry; Physical Oceanography

INSTITUTES - Each of the following Institutes, which foster interdisciplinary research addressing critical issues, will award a scholarship to support related research: Ocean and Climate Change Institute; Coastal Ocean Institute; Deep Ocean Exploration Institute; Ocean Life Institute

The NOAA-WHOI Cooperative Institute for the North Atlantic Region (CINAR) will award a Fellowship in one of five theme areas: Ecosystem Forecasting, Ecosystem Monitoring, Ecosystem Management, Protection and Restoration of Resources, Sustained Ocean Observations and Climate Research.

Awards are competitive, with primary emphasis placed on research promise. Scholarships are for 18-months with an annual stipend of \$56,000, a modest research budget and eligibility for group health and dental insurance. Recipients are encouraged to pursue their own research interest in association with resident Scientific and Senior Technical Staff. Communication with potential WHOI advisors prior to submitting an application is encouraged.

Further information, application forms, and links to the Individual Departments, Institutes and Centers and their research themes may be obtained at: <http://www.whoi.edu/postdoctoral>, or by contacting the Postdoctoral Fellowship Committee at: (508) 289-2950, or postdoc@whoi.edu. EOE/AEE

Advanced Electron-Nuclear Magnetic Resonance Spectroscopies Faculty Position at Penn State University

The Department of Biochemistry and Molecular Biology and the Department of Chemistry at The Pennsylvania State University jointly seek applicants for a tenure-track faculty position in the area of advanced electron-nuclear magnetic resonance (ENDOR, ESEEM, PELDOR, HYSCORE) spectroscopies and their applications to biological and chemical research problems. The successful candidate will evidence the abilities to establish a nationally and internationally recognized research program and to teach lecture and laboratory courses in the areas of biophysics and biochemistry at both the undergraduate and graduate levels. Evidence of past research accomplishments in the form of a strong publication record is considered essential. Preference will be given to candidates who demonstrate strength in theoretical and technical development of these methods in addition to their applications to important research problems. Candidates should send cover letter, curriculum vitae, list of publications, summary of research accomplishments, and statement of future research interests to **Ms. Jean Brooks** as PDF files to jobs@bmb.psu.edu. Please reference job number **33275-D** on your cover letter and arrange for three letters of evaluation to be sent to the same address. Review of applications will begin on **November 10, 2010**, and will continue until the position is filled.

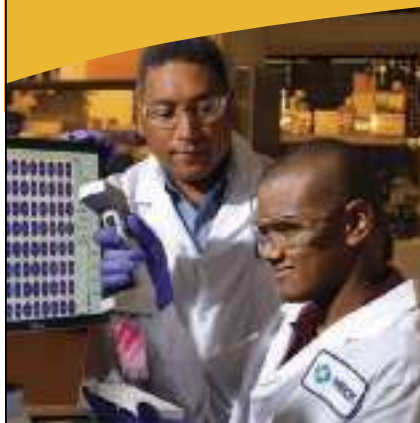
Penn State is committed to Affirmative Action, Equal Opportunity and the diversity of its workforce.

FELLOWSHIPS

Science Scholarships and Fellowships

UNCF/MERCK SCIENCE INITIATIVE

The UNCF/Merck Science Initiative is an innovative approach that creates opportunities in the biological, chemical and engineering sciences for African American students throughout the country.



UNDERGRADUATE

Science Research Scholarship Awards

- Scholarships up to \$25,000
- A paid summer internship at a Merck facility with stipend totaling more than \$5,000
- Mentoring and networking opportunities
- Eligibility: College juniors, science or engineering majors, 3.3 GPA

GRADUATE

Science Research Dissertation Fellowships

- Fellowships up to \$53,500
- Mentoring and networking opportunities
- Eligibility: Ph.D. or equivalent degree candidates engaged in dissertation research in the biological, chemical or engineering fields

POSTDOCTORAL

Science Research Fellowships

- Fellowships up to \$92,000
- Mentoring and networking opportunities
- Eligibility: Ph.D. or equivalent degree recipients in the biological or chemical research fields

APPLY ON-LINE

<http://umsi.uncf.org>
Submit by December 1, 2010

T 703 205 3400

F 703 205 3550

E uncfmerck@uncf.org



GENERAL ELIGIBILITY REQUIREMENTS:
Must be African American and a U.S. citizen or a permanent resident



MANHATTAN COLLEGE

Dean of the School of Science

Manhattan College seeks a dynamic, innovative, and collaborative leader to assume the position of Dean of the School of Science.

Overlooking Van Cortlandt Park in the Riverdale section of New York City, Manhattan College is an independent Catholic institution of higher learning that embraces qualified women and men of all faiths, races, and ethnic backgrounds. Established in 1853, the College is founded upon the Lasallian tradition of excellence in teaching, respect for individual dignity, and commitment to social justice inspired by the innovator of modern pedagogy, John Baptist de La Salle.

Currently, the College has a student body of approximately 3,250: 2,900 undergraduates and 350 graduate students.

The School of Science is one of five schools, together with Arts, Business, Education, and Engineering, that make up the College. The School currently has majors in biology, biochemistry, chemistry, computer science, mathematics, and physics. At the present time there are no graduate programs in the school, although some are being considered. The science faculty consists of a diverse group of 37 full time members dedicated to teaching excellence and scholarly activity. The School of Science has a rich tradition of involving students in undergraduate research projects leading to conference presentations and publications.

The Dean will lead and support the faculty in the development of interdisciplinary programs and innovative concentrations with the goal of increasing both the number and the quality of students enrolling in the School of Science. She/he will encourage the writing of grant proposals for funding the purchase of scientific equipment and the continuation and expansion of faculty/student research projects. The Dean will provide a strong voice for the sciences both within the College community and to outside constituencies. She/he will work to expand collaborative efforts among the faculty in the School of Science as well as with faculty from the other schools of the College.

The successful candidate will be a proven administrator with a strong record of promoting excellence in science education. He/she will have experience in curriculum development, innovative pedagogy, student support services and advising, grant writing, and undergraduate research. A Ph.D. in one of the science disciplines and a proven record of excellence in teaching and research, appropriate for a tenured member of one of the departments in the school are expected. She/he should give evidence of the ability to provide leadership in the recruitment and retention of highly qualified students and faculty. The Lasallian tradition is at the core of the College's mission, and the successful candidate will be an advocate for those educational values and possess a track record of helping students to integrate science education and a strong core curriculum in the School of Arts.

Please send cover letter, resume, list of five references and salary history or requirements by **December 3, 2010**, to: **Barbara Fabé, Vice President for Human Resources, Manhattan College, 4513 Manhattan College Parkway, Riverdale, NY 10471**. All submissions must be emailed only. Please send all e-mail to patricia.stone@manhattan.edu. Women and Minorities are encouraged to apply. We are committed to a diverse campus community. AA/EOE/M/F/D/V Please refer to our website www.mancol.edu/hrs/jobs/index.shtml



HARVARD UNIVERSITY Assistant/Associate Professors Department of Genetics and Complex Diseases

The Department of Genetics and Complex Diseases (GCD) at the Harvard School of Public Health (HSPH) invites applications for tenure-track positions at the level of assistant professor. Exceptional associate professor candidates will also be considered. Successful applicants will hold a PhD and/or MD degree and will have a record of outstanding productivity. Individuals are sought particularly in the following areas to complement the existing research and training goals of the department: signal transduction related to energy and nutrient sensing pathways, regulation of metabolic homeostasis, inflammatory and stress response pathways related to chronic metabolic diseases and aging, cancer metabolism, and epigenetic regulation of metabolism. Individuals using systems and/or computational approaches applied at a mechanistic level to problems of metabolic homeostasis, gene-environment interactions and/or adaptive responses are also encouraged to apply. The candidate should possess the ability to work collaboratively with other scientists and the scholarly qualities required to mentor doctoral students in the graduate program in the Division of Biological Sciences. Generous startup packages and state-of-the-art research facilities are available.

Please send a letter of application, including a statement of current and future research interests, curriculum vitae, sample publications, and the names of four references to the following address. Applicants should ask their four references to write independently to this address.

Chair, GCD Search, c/o Holly Johnsen
Department of Genetics & Complex Diseases
Harvard School of Public Health
655 Huntington Avenue, Building II, 101
Boston, MA 02115
gcddept@hsph.harvard.edu

The Harvard School of Public Health is committed to increasing the representation of women and minorities in its faculty, and encourages applications from such candidates.

POSITIONS OPEN



PLANT EVOLUTIONARY BIOLOGIST

The Department of Biology invites applications for a faculty position in the area of plant evolutionary biology. We are especially interested in individuals whose research have a field component and use molecular techniques to address fundamental questions in plant evolutionary biology. Primary teaching responsibilities will include related undergraduate and graduate courses. This is a nine-month, tenure-track position at the rank of **ASSISTANT PROFESSOR**. Anticipated start date is August 16, 2011, pending funding. The successful applicant will be expected to develop a productive, externally funded research program and direct graduate students through the Ph.D. level. Postdoctoral experience is expected and demonstrated evidence of excellence in scholarship and teaching is required. Candidates must have demonstrated experience working in and fostering a diverse faculty, staff, and student environment or commitment to do so as a faculty member at VCU. Competitive startup funds and excellent core facilities are available.

Virginia Commonwealth University has an enrollment of 32,000 students, including over 1,600 undergraduate and nearly 100 graduate students in Biology. The Department of Biology ([website: http://www.has.vcu.edu/bio](http://www.has.vcu.edu/bio)) has 32 faculty members with diverse research interests in the following three areas of excellence: Cell Regulation, Evolution, and Ecological Processes and Applications. Additional research opportunities are available through the Center for Environmental Studies ([website: http://www.vcu.edu/cesweb/](http://www.vcu.edu/cesweb/)) and at the Rice Center, VCU's nearby field station on the James River ([website: http://www.vcu.edu/rice/](http://www.vcu.edu/rice/)).

Submit curriculum vitae, statements of research and teaching interests, and three letters of reference by December 1, 2010 to: **Stephanie Millican, Department of Biology, Virginia Commonwealth University, Richmond, VA 23284-2012.**

Virginia Commonwealth University is an Equal Opportunity/Affirmative Action Employer. Women, minorities and persons with disabilities are encouraged to apply.

POSTDOCTORAL POSITIONS in Structural Biology Harvard Medical School

Funded postdoctoral research positions are available immediately to work in the Molecular Medicine Laboratory and Macromolecular Crystallography Unit, at the Beth Israel Deaconess Medical Center, Harvard Medical School. Ongoing projects include the structural analysis of human proteins and nucleoprotein complexes that play important roles in the pathogenesis of cancer, tuberous sclerosis, Alzheimer's disease, schizophrenia, drug abuse, and AIDS. (i) Position in Macromolecular Crystallography: experience in protein and/or nucleic acid X-ray crystallography using MIR and/or MAD techniques is required; (ii) Position in Protein Biochemistry: experience in protein expression and purification using bacterial and/or baculovirus expression systems is required. Send curriculum vitae and three reference letters to: **Dr. John A.A. Ladias, Research North 325, 99 Brookline Avenue, Boston, MA 02215. E-mail: jladias@bidmc.harvard.edu. An Equal Opportunity/Affirmative Action Employer.**

ASSISTANT PROFESSOR of BIOLOGY—The Biology Department of Whitworth University invites applications for a tenure-track position as Assistant Professor of Biology with a primary focus on Environmental Biology, Field Biology, Wildlife Biology, and/or Conservation Biology. Earned Doctorate in an appropriate area of biology or environmental science by August 2011, and demonstrated teaching ability required. Go to [website: http://www.whitworth.edu/jobs](http://www.whitworth.edu/jobs) for further details and information on how to apply.

POSITIONS OPEN



SPATIAL/LANDSCAPE ECOLOGIST

The Department of Biology invites applications for a quantitative spatial/landscape ecologist. We are especially interested in applicants whose research integrates field work, theory, statistical and mathematical modeling, and/or GIS/remote sensing to study organismal, population or community level processes at broad spatial scales. Primary teaching responsibilities will include related undergraduate and graduate courses. This is a nine-month, tenure-track position at the rank of **ASSISTANT PROFESSOR**. Anticipated start date is August 16, 2011, pending funding. The successful applicant will be expected to develop a productive, externally funded research program and direct graduate students through the Ph.D. level. Postdoctoral experience is expected and demonstrated evidence of excellence in scholarship and teaching is required. Candidates must have demonstrated experience working in and fostering a diverse faculty, staff, and student environment or commitment to do so as a faculty member at VCU. Competitive startup funds are available.

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Submit curriculum vitae, statements of research and teaching interests, and three letters of reference by December 1, 2010 to: **Stephanie Millican, Department of Biology, Virginia Commonwealth University, Richmond, VA 23284-2012.**

Virginia Commonwealth University is an Equal Opportunity/Affirmative Action Employer. Women, minorities and persons with disabilities are encouraged to apply.

DEPARTMENT HEAD Department of Biology Utah State University

The College of Science at Utah State University seeks applications for the position of Head, Department of Biology. Applicants must have a Ph.D. or equivalent in the biological sciences, or a related field, and an excellent record of academic and leadership experience and research productivity at a teaching and research institution. The department has built a national reputation for collaborative and innovative research across the breadth of the life sciences and promotes effective learning and research opportunities for our students. Applicants should submit curriculum vitae, a statement of leadership and management experiences and philosophy, and vision for the future of the department, as well as the names and e-mail addresses of three references on-line at [website: http://www.usu.edu/science](http://www.usu.edu/science). Evaluation of applicants will begin January 21, 2011, and will continue until the position is filled. For further information, please visit our [website: http://www.usu.edu/science](http://www.usu.edu/science).

Utah State University is an Equal Opportunity/Affirmative Action Employer committed to assembling a diverse faculty. Women and members of minority groups are strongly encouraged to apply. Logan is nestled in a mountain valley 80 miles north of Salt Lake City, Utah. Opportunities for a wide range of outdoor activities are plentiful.

POSITIONS OPEN



NEUROBIOLOGIST

The Department of Biology invites applications for a molecular and cellular biologist with expertise in the mechanisms of neural diseases or neural development. The successful applicant will join a dynamic group of investigators in Biology and departments in VCU Life Sciences and on the medical campus of VCU who investigate fundamental disease and developmental processes in a variety of organisms. Primary teaching responsibilities will include related undergraduate and graduate courses. This is a nine-month, tenure-track position at the rank of **ASSISTANT PROFESSOR**. Anticipated start date is August 16, 2011, pending funding. The successful applicant will be expected to develop a productive, externally funded research program and direct graduate students through the Ph.D. level. Postdoctoral experience is expected and demonstrated evidence of excellence in scholarship and teaching is required. Candidates must have demonstrated experience working in and fostering a diverse faculty, staff, and student environment or commitment to do so as a faculty member at VCU. Competitive startup funds and excellent core facilities are available.

Virginia Commonwealth University has an enrollment of 32,000 students, including over 1,600 undergraduate and nearly 100 graduate students in Biology. The Department of Biology ([website: http://www.has.vcu.edu/bio](http://www.has.vcu.edu/bio)) has 32 faculty members with diverse research interests in the following three areas of excellence: Cell Regulation, Evolution, and Ecological Processes and Applications.

Submit curriculum vitae, statements of research and teaching interests, and three letters of reference by December 1, 2010 to: **Stephanie Millican, Department of Biology, Virginia Commonwealth University, Richmond, VA 23284-2012.**

Virginia Commonwealth University is an Equal Opportunity/Affirmative Action Employer. Women, minorities and persons with disabilities are encouraged to apply.

ASSISTANT PROFESSOR

The Department of Environmental Science, Loyola University Chicago (LUC), seeks to fill a tenure-track Assistant Professor position, for academic year 2011–2012, pending approval of funding. The department collaborates with LUC's Center for Urban Environmental Research and Policy the larger efforts of Loyola to facilitate environmental sustainability at LUC and in the local community.

Candidates must have a Ph.D. in the environmental sciences; research expertise linking science to policy, management, or economics; and proven ability to publish work that integrates disciplines. Ideal candidates will have taught at the University level, be able to secure external research funding, and involve students in research program. Teaching expectations include Environmental Sustainability, existing physical or life science courses for non-science majors, and a newly developed course for majors that enhances the existing curriculum.

To apply, submit curriculum vitae and a letter of interest addressing teaching philosophy and research agenda to [website: http://www.careers.luc.edu](http://www.careers.luc.edu), and arrange for electronic submission of three letters of recommendation to the address above. Applications should be received by December 15, 2010.

Loyola University Chicago, Chicago's Jesuit Catholic University, is an Equal Opportunity/Affirmative Action Employer with a strong commitment to diversifying its faculty. Applications from women and minority candidates are especially encouraged.

Terumo Life Science Foundation

財団法人 テルモ科学技術振興財団

Terumo Life Science Foundation announces the establishment of the Terumo International Prize – An academic award for biomaterials researchers –

In recent years, combined research in the fields of biomaterials and regenerative medicine is accelerating rapidly, and various efforts toward practical application are being made. The fusion of excellence in materials engineering, life science, and the related discipline of systems engineering is essential for this area. This Prize shall be awarded to outstanding researchers who have demonstrated unique, internationally renowned achievements in research, made significant contributions to the field of regenerative medicine particularly through novel biomaterials discovery, and continued to work in the frontline of research.

We hope that this Prize will be a great source of encouragement to researchers, resulting in the further evolution of research towards practical application and a brighter future for humankind.

Details of the Prize:

The Terumo International Prize shall be awarded to one outstanding researcher. The awardee will receive a certificate and a commemorative item as the main prize, together with a supplementary monetary prize of 10 million yen.

Application method:

An application in the form of a recommendation shall be filed. (No self-recommendation accepted)

Please fill out the application form and submit it to the Secretariat along with all the required documents. The application can be made online or by post. Please refer to the official Web site for more details.

Application period:

to December 24, 2010

Application by post must be postmarked on or before the above deadline. Online application will close at 5:00 PM (Japanese Standard Time) of the last day.

**NOW ACCEPTING
APPLICATIONS**

**Official
Website**

<http://www.terumozaidan.or.jp/english/>

POSITIONS OPEN



BIG DREAMS. BOLD FUTURE.

ASSISTANT/ASSOCIATE PROFESSOR

Department of Molecular Medicine

We are seeking candidates for a tenure-track position at the rank of Assistant or Associate Professor, who preferably employ molecular approaches addressing problems related to metabolic disorders such as obesity, metabolic syndrome and diabetes, and which complement existing strengths in the Department. The Department is located in a vibrant and expanding USF Health Complex on the main campus of the University of South Florida that includes the Colleges of Medicine, Nursing, and Public Health, the H. Lee Moffitt Comprehensive Cancer Center and Research Institute, and the Byrd Alzheimer's Institute. USF is a nationally recognized research campus with extramural grants and contract activity in excess of \$350 million annually and is home to several Centers for Excellence in Biomedical Research and state-of-the-art facilities. Over the past 3 years, the state of Florida committed an additional \$450 million to the existing medical research budget, and as a result, USF is now part of a collaborative research corridor running through the heart of the sunshine state.

Successful candidates for this position are expected to develop and maintain a competitively funded research program, and to participate in medical and graduate education. To support these expectations, the Department offers strong institutional salary support, competitive start-up packages, excellent core facilities, and a collegial atmosphere to create an environment that promotes success. Minimum requirements for an Assistant Professor position include a MD, PhD, or MD/PhD with at least two years postdoctoral experience. Appointment at the Associate Professor level requires a minimum of five years experience at the Assistant professor level or equivalent, nationally-recognized and federally funded research program and experience mentoring graduate students.

All applications must be submitted on-line by going to (<https://employment.usf.edu/applicants/jsp/shared/position/JobDetails.css.jsp?postingId=151861>) and uploading a cover letter, curriculum vitae, research plan, and statement of teaching philosophy. Separately, please request three letters of reference to be sent by mail or e-mail to Dana Cole at dcole@health.usf.edu. Review of applicants will begin on November 15, 2010 and will continue until the position is filled.

Dr. Duane Eichler, Chair Search Committee, Department of Molecular Medicine, University of South Florida-Health, 12901 Bruce B. Downs Blvd. MDC7, Tampa, Florida 33612-4799, http://health.usf.edu/nocms/research/molecular_medicine/

The University of South Florida is an Equal Opportunity / Affirmative Action / Equal Access Institution. For disability accommodations, contact Dana Cole at (813) 974-8320 - a minimum of 5 working days in advance. According to Florida law, search records, including applications and search committee meetings, are open to the public.



AWARDS



HUMAN FRONTIER SCIENCE PROGRAM (HFSP)

CALL FOR NOMINATIONS FOR THE 2011 HFSP NAKASONE AWARD

In keeping with its mission to stimulate innovative international research, HFSP invites nominations for an annual award highlighting frontier contributions in the life sciences. This award recognizes the vision of former Prime Minister Nakasone of Japan in the creation of HFSP. Typically these will be conceptual breakthroughs for investigating the complex mechanisms of living organisms which have important consequences for scientists throughout the world. Both theoretical and methodological contributions are eligible.

The winner of the 2010 award was Karl Deisseroth of Stanford University, for his pioneering work in the development of optogenetic techniques for understanding complex neuronal circuits in the brain.

The competition is open; it is not limited to HFSP awardees and there is no age limit for candidates. However the jury will pay particular attention to recent breakthroughs by younger scientists. Nominations should be made before Monday December 6th using the standard one-page nomination form (<http://bit.ly/bjaCLE>). Nominations from 2010 should not be resubmitted. The selection will be made by the HFSP Council of Scientists and the award announced at the end of March.

The awardee will receive an unrestricted research grant of 10,000 USD, a commemorative medal and will be expected to deliver a plenary lecture at the HFSP annual awardees meeting (in Montreal, Canada, June 5-8, 2011).

Please see our web site www.hfsp.org for further information

HFSP, 12 quai Saint-Jean, 67080 STRASBOURG Cedex, FRANCE

POSITIONS OPEN

PROFESSOR AND DIRECTOR DIABETES CENTER

The University of North Carolina at Chapel Hill

The School of Medicine at the University of North Carolina at Chapel Hill seeks a dynamic investigator to provide effective academic and administrative leadership for the recently established Diabetes Center. The purpose of the center is to promote, facilitate, and coordinate research and training in diabetes across this highly collaborative campus with over \$800 million in research funding. The position reports to the Dean of the School of Medicine.

Chapel Hill is a dynamic community located within the Research Triangle, one of the largest concentrations of biomedical scientists in the world. Mild winters, a multitude of arts and cultural programs, vibrant night-life, and nearby oceans and mountains create a wonder environment to live and work.

Though there are approximately 200 investigators with an interest in diabetes, the institution is committed to additional recruitment to complement existing expertise and to create a premiere diabetes research enterprise across the spectrum from basic and population research to clinical and community based investigation including entrepreneurial activity and commercialization.

Minimal qualifications for this position include an M.D. and/or Ph.D. degree, a distinguished record of scientific research and funding, and evidence of administrative skills. Consideration of candidates will begin immediately and continue until the search is concluded. Interested individuals should apply online at [website: http://jobs.unc.edu/2500493](http://jobs.unc.edu/2500493) and include a cover letter, current curriculum vitae, and contact information for four references.

For questions or additional information regarding the position, you can contact:

John Buse, M.D., Ph.D., Chair,
Diabetes Center Director Search Committee
Office of the Dean, School of Medicine
University of North Carolina at Chapel Hill
CB# 7000, 4060 Bondurant Hall
Chapel Hill, NC 27599-7000
Attn: Carol Ashby
E-mail: carol_ashby@med.unc.edu

UNC Chapel Hill is an Equal Opportunity Employer.

FACULTY POSITION ASSISTANT PROFESSOR

Integrative Vertebrate Physiology
University of California, Riverside

The Department of Biology at the University of California, Riverside invites applications for a tenure-track, 9-month academic position at the Assistant Professor rank beginning July 1, 2011. A Ph.D. in Physiology or a related field and at least one year of postdoctoral experience are required. The salary for this position is commensurate with the education and experience of the successful candidate. Applicants are expected to develop a strong and externally funded research program in functional biology of vertebrates, focusing on physiological mechanisms, integrative functioning of organ systems, biomechanics, and/or behavior. We seek a candidate who can bridge our existing departmental strengths in integrative and comparative physiology with campus-wide strengths in biomedical and applied physiology.

Opportunities include collaborations with a variety of campus research centers ([website: http://www.cnas.ucr.edu/centers/index.html](http://www.cnas.ucr.edu/centers/index.html)), the emerging medical school, and the University of California Natural Reserve System. All faculty members teach at the undergraduate and graduate level, and may participate in one or more interdepartmental graduate programs. Applicants should log on to [website: https://academicjobsonline.org/ajob/jobs/574](http://academicjobsonline.org/ajob/jobs/574) to submit their curriculum vitae, separate statements of research and teaching interests, and up to three reprints. In addition, applicants should request that three letters of recommendation be submitted through [website: https://academicjobsonline.org/ajob/jobs/574](http://academicjobsonline.org/ajob/jobs/574).

Review of applications will begin on November 30, 2010. [Website: http://biology.ucr.edu](http://biology.ucr.edu).

The University of California is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN

UNIVERSITY OF CALIFORNIA, IRVINE Chemical Engineering and Materials Science

The Henry Samueli School of Engineering at the University of California, Irvine invites qualified applicants for a faculty position in the Department of Chemical Engineering and Materials Science. Candidates should have a Doctoral degree in Materials Science and Engineering, Chemical Engineering, or related discipline, demonstrated excellence in research, and a strong commitment to teach courses at undergraduate and graduate levels. Outstanding candidates whose research programs impact the areas of biomaterials and bio-inspired materials are particularly encouraged to apply. Broadly, this includes (but is not limited to) the development and application of materials in emerging interfaces between healthcare and nano-devices. Candidates qualified to teach undergraduate courses in materials science and engineering are preferred.

Nominally, the position will be at the **ASSISTANT PROFESSOR** level (tenure-track), but in exceptional cases senior applicants will be considered. The successful candidate will be considered for a Samueli Faculty Career Development Professorship (junior level) or a Samueli Endowed Chair (senior level).

Please submit applications electronically at [website: http://www.eng.uci.edu/employment/faculty](http://www.eng.uci.edu/employment/faculty).

Candidates should submit their curriculum vitae, a list of publications, descriptions of research plans and teaching interests, and names of three references. Selection will begin December 1, 2010, and will continue until the position is filled. For additional information, please contact: **Janine Le, Department of Chemical Engineering and Materials Science, University of California, Irvine, 916 Engineering Tower, Irvine, CA 92697-2575. E-mail: jhle@uci.edu, telephone: (949) 824-3786.**

UCI is an Equal Opportunity/Affirmative Action Employer committed to excellence through diversity and strongly encourages applications from all qualified candidates including women and minorities. UCI is responsive to the needs of dual career couples, is dedicated to work-life balance through an array of family-friendly policies, and is the recipient of an NSF ADVANCE Award for gender equity, a program recently expanded at UC Irvine to include racial/ethnic equity.

OPEN-RANK TENURE-TRACK FACULTY POSITION IN PLANT STRESS BIOLOGY Division of Plant Sciences University of Missouri

The Division of Plant Sciences and the Interdisciplinary Plant Group, University of Missouri, Columbia (MU), invite applications for an open-rank tenure-track position in Plant Stress Biology, a major theme within the broader MU emphasis area "Plants for Changing Environments." We seek outstanding candidates using modern methods to address research challenges in the areas of biotic stress from disease, insect herbivory, and other sources; and/or abiotic stress from drought or other extreme environmental conditions.

The successful candidate will be expected to establish an active, extramurally funded research program in their area of research specialization. The candidate will also be expected to contribute to the research and teaching missions of the Division of Plant Sciences and of the campus-wide collaborative Interdisciplinary Plant Group ([website: http://ipg.missouri.edu](http://ipg.missouri.edu)). The applicant must have a Ph.D. degree in Plant Sciences, Biochemistry, Ecology, Plant Pathology, Nematology, Entomology, or related field. For more information on the position and on research areas of particular interest to the Division of Plant Sciences, see [website: http://plantsci.missouri.edu/](http://plantsci.missouri.edu/).

To ensure full consideration, applications should be submitted by December 5, 2010; however, applications will be considered until the position is filled. Applicants must submit curriculum vitae, and a concise summary of research and teaching interests and future plans. These materials should be submitted electronically as a single PDF file to [e-mail: plantsci@missouri.edu](mailto:plantsci@missouri.edu). Applicants should also arrange for three letters of recommendation to be sent electronically to the same address. Questions regarding this position should be directed to **Dr. Robert Sharp at e-mail: sharpr@missouri.edu, telephone: 573-882-1841.**

MU is an Equal Opportunity/Affirmative Action Employer. Women and minorities are especially encouraged to apply. EOE/ADA/VTY.

NEUROSCIENCE

FACULTY POSITION IN NEUROSCIENCES University of New Mexico School of Medicine

The Department of Neurosciences invites applications for a tenure-track faculty position at the **ASSISTANT PROFESSOR** level. Minimum Requirements: Applicants must have a Ph.D. or M.D. degree, or the equivalent, with postdoctoral experience. Preferences: Applicants with an independent research program, or strong potential for obtaining extramural funding, as evidenced by high quality publications; Neuroscientists with primary research interests that build upon department strengths in molecular, cellular and behavioral aspects of neurodevelopment, psychiatric or neurodegenerative disorders; Experience or interest in participating in graduate and medical school teaching programs. For more details on current research activities in the Department of Neurosciences see [website: http://www.unm.edu/~neurohsc](http://www.unm.edu/~neurohsc). Opportunities exist for collaborations with faculty in other basic science and clinical departments in the Health Sciences Center ([website: http://hsc.unm.edu/som/departments.shtml](http://hsc.unm.edu/som/departments.shtml)), the Department of Psychology, the MIND Research Network, and Sandia and Los Alamos National Laboratories. The position includes a competitive state-funded salary, research startup funds, and research facilities. The selected individual should have experience or interest in participating in graduate and medical school teaching programs. For best consideration, applications must be received by December 10, 2010; however, position will remain open until filled. UNM's confidentiality policy ("Disclosure of Information about Candidates for Employment," UNM Board of Regents' Policy Manual 6.7), which includes information about public disclosure of documents submitted by applicants, is located at [website: http://www.unm.edu/~brpm/r67.htm](http://www.unm.edu/~brpm/r67.htm). For complete details of this position and to apply, access Faculty Postings at [website: https://unmjobs.unm.edu/](https://unmjobs.unm.edu/). Reference Posting # 0808282. The University of New Mexico is an Equal Opportunity/Affirmative Action Employer and Educator.

POSITIONS OPEN

THE HEISER PROGRAM FOR RESEARCH IN LEPROSY AND TUBERCULOSIS

POSTDOCTORAL RESEARCH FELLOWSHIPS in leprosy and tuberculosis research available at a stipend level of \$40,000 a year for two years. Research Grants in leprosy usually in amounts up to \$50,000 for one year.

Applicants should have M.D., Ph.D., or equivalent degree. Application deadline March 1, 2011, for awards to be activated August through December, 2011. For information and forms [website: http://www.NYCommunitytrust.org](http://www.NYCommunitytrust.org). Click on Grant Seekers and select "Requests for Proposals" from the drop-down menu. Click on The Heiser Program in the list down the right side of your screen to access the full RFP and application forms. For questions or problems, [e-mail: lm@nyct-cfi.org](mailto:lm@nyct-cfi.org).

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